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**Kentucky Academy of Sciences
Business Meeting
Murray State University
5 November 2011**

Outgoing President's Report

Barbara Ramey called the meeting to order at 5:30 P.M. and welcomed the 37 KAS members present. She stated the KAS Governing Board approved Mr. Martin Matisoff as the new Editor of the JKAS. She thanked Dr. David White for his service to KAS during his tenure as the JKAS Editor. Dr. Ramey shared the response to the Natural History Museum Symposium had been positive and Dr. Albert Meier had expressed an interest in initiating a Natural History Survey. Dr. Ramey also mentioned 2014 is the Centennial Celebration of KAS and asked for volunteers to serve on the Centennial Committee. Dr. Cathleen Web volunteered to serve.

Treasurer's Report (Hand out filed with minutes)

Ken Crawford reported that KAS account balances are currently elevated due to the receipt of the meeting revenue but will return to normal levels upon payment of meeting expenses. Dr. Crawford stated the proposed 2012 KAS budget estimates \$103,700 in revenues and \$103,750 in expenses. The 2011 Athey Trusts distributions due to the current economic environment have been below projections.

Ad Hoc Constitution Committee

Eric Jerde reviewed proposed changes to the KAS Constitution. The main focuses of

the updates are inclusion of enhanced affiliates and electronic communications. There was no discussion and the proposed changes were unanimously approved by the KAS membership.

Elections

Sean Reilley reported election results, which were tabulated using Survey Monkey this year:

Vice President-KC Russell, Treasurer-Ken Crawford, Secretary-Robert Kingsolver, Biological Science- Pamela Feldhoff, and At large Representative- KatieAnn Skogsberg.

Recognition of Board members completing terms in office:

Dick Durtsche, Rob Kingsolver, Ken Crawford, and KatieAnn Skogsberg were both thanked for their service and congratulated for their respective re-elections to the Board. Outgoing President Ramey presented the gavel and the customary donation to the President's fund to Incoming President Dawn Anderson.

New President's Remarks

Dawn Anderson thanked the outgoing President and expressed gratitude to Barb Ramey for dedication to KAS.

Meeting Adjourned

An Annotated Checklist of the Caddisflies (Insecta: Trichoptera) of Kentucky

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ABSTRACT

Distributional records for 293 caddisfly species representing 22 families and 68 genera are reported from Kentucky along with information on taxonomy, flight period, habitat, and conservation status. Sixty-nine species represent new records for the Commonwealth. Kentucky's geographic regions are compared with respect to species richness. Distributions are summarized for all species; detailed occurrence data are provided for new records, species with limited distributions, and those representing substantial range extensions. A total of 69 species (24% of the fauna) are identified as imperiled or vulnerable within Kentucky.

INTRODUCTION

One of the largest and most diverse groups of aquatic insects is the order Trichoptera, commonly known as caddisflies. At least 13,574 species have been described worldwide (Morse 2011), including over 1600 species from North America (Morse 1993). Adult caddisflies are small- to medium-sized (2–30 mm), holometabolous insects that resemble moths in general appearance (Ross 1944). The majority of adults tend to be cryptically-colored (shades of gray or brown), but a few species exhibit bright colors and elaborate wing patterns. The adults are generally inactive during the day, concealing themselves in crevices or foliage, but they

become very active at dusk, sometimes producing large, synchronous emergences.

With few exceptions, the caterpillar-like larvae are strictly aquatic and occupy a wide variety of lentic and lotic habitats. Some species are free-living predators, others construct and occupy silken tubes that are used to filter organic particles from the water column, and others construct and occupy tubular, portable cases composed of plant or mineral fragments. Most case-making larvae graze on diatoms or fine organic particles attached to rock or plant surfaces while others shred dead plant material (leaves and small wood fragments) supporting growths of fungi and bacteria (Wiggins 1996).

As a result of their varied feeding strategies, caddisfly larvae play an important ecological role in the cycling of nutrients in aquatic systems, especially in lotic environments

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(Wiggins and MacKay 1978; Benke and Wallace 1980; Ross and Wallace 1983). Larvae and adults also serve as prey items for a variety of other organisms, including fishes (Lotrich 1973); amphibians (Petraska 1998; Davic and Welsh 2004), birds (Crichton 1959; Todd et al. 1998), and bats (Brack and LaVal 1985; Kurta and Whitaker 1998). Larval caddisflies have become an important component of biological assessment programs due to their sensitivity to water quality and habitat degradation (Rosenberg and Resh 1993), and some species have been recognized by state and federal agencies as rare or at-risk species of conservation interest (Harris 1990; Rasmussen 2008; KSNPC 2010). With regard to public interest, caddisflies are most familiar to trout fishermen and other anglers, who have spent considerable time and effort in duplicating the appearance of caddisfly larvae, pupae, and adults and mimicking their behaviors in lure presentations. Numerous books have been published instructing anglers on caddisfly identification, behavior, and imitations (LaFontaine 1981; Pobst and Richards 1998).

Faunistic surveys such as the present study are valuable to water quality managers, resource agencies, and other scientists because (*i*) they provide baseline data that can be used in monitoring temporal changes in environmental quality (Resh 1975); (*ii*) they provide new distributional information that can be used to strengthen biomonitoring indices (Rosenberg and Resh 1993); (*iii*) they allow for the evaluation of distribution patterns and formulation of speciation hypotheses (Rosenberg and Resh 1993); (*iv*) they document regional biodiversity, including the presence of rare or at-risk species (Houghton et al. 2001; DeWalt et al. 2005); and (*v*) they provide baseline data that resource agencies can use to evaluate the conservation status of imperiled species.

The primary objective of our study was to compile an updated and accurate list of Kentucky's caddisflies by surveying sites across the Commonwealth and concentrating our efforts in areas not previously sampled. We also wanted to document new Kentucky records, establish general distributional patterns for all species, and contribute ecological information (e.g., habitat preference, flight period) that is lacking for many species.

Finally, we wanted to evaluate the conservation status of Kentucky's caddisflies and develop a new, updated list of imperiled or vulnerable taxa.

HISTORICAL COLLECTIONS

In his classic work on the caddisflies of Illinois, Ross (1944) provided Kentucky distributional records for 46 species. Following his publication, species descriptions and ecological studies by Ross (1959), Minckley (1963), Minshall (1968), Ross and Yamamoto (1965), Etnier (1973), Resh and Haag (1973), and Resh (1974) yielded an additional 42 Kentucky records.

Resh (1975) published the first and only checklist of Kentucky Trichoptera, providing records for 158 species. About one-third of these species were obtained during biological investigations for a proposed reservoir (Taylorsville Lake) in the Salt River basin of central Kentucky (Resh 1975; Neff and Krumholz 1973). Other records came from surveys by the Water Resources Laboratory (WRL) at the University of Louisville and examination of entomological collections from the University of Louisville, University of Kentucky, The University of Tennessee, and the Illinois Natural History Survey. Collection records were concentrated in five general areas: the eastern edge of Land Between the Lakes National Recreation Area (LBL) in western Kentucky (Christian, Lyon, and Trigg counties), the Daniel Boone National Forest (DBNF) and Lake Cumberland region in south-central Kentucky (McCreary and Wayne counties), the Lexington area in central Kentucky (Fayette and Jessamine counties), the Robinson Forest area in eastern Kentucky (Breathitt County), and the Levisa Fork basin near Kentucky's eastern border with West Virginia (Johnson County). Caddisfly records from other large, biologically significant areas of the Commonwealth (e.g., Green River basin, Licking River basin, and western Kentucky) were lacking. Resh (1975) commented that these gaps would need to be filled before the distribution of Kentucky caddisflies could be described accurately.

Over the last 35 years, some of these gaps were addressed by Picazo and DeMoss (1980), Thoeny and Batch (1983), Haag and Hill (1983), Haag et al. (1984), Parker and Wiggins

(1987), Phillippi and Schuster (1987), Floyd and Schuster (1990), Floyd (1992), Houp and Schuster (1997), Houp et al. (1998), Houp (1999), Etnier et al. (2006), and Etnier et al. (2010), who added 66 species to Kentucky's fauna. Unfortunately, most of these studies covered only limited portions of the state and did not fully address the data gaps noted by Resh (1975).

Kentucky's previously reported fauna of 224 species is comparable to that of Arkansas, 176 species (Bowles and Mathis 1989; Moulton and Stewart 1996); Indiana, 190 (Waltz and McCafferty 1983); Illinois, 183 (Ross 1944); Missouri, 155 (Mathis and Bowles 1992; Moulton and Stewart 1996); and West Virginia, 193 (Tarter 1990; Tarter et al. 1999); however, the fauna is considerably smaller than Ohio's 270 species (Huryn and Foote 1983; Usis and Foote 1989; Armitage et al. 2011); Tennessee's 352 species (Etnier et al. 1998; DeWalt and Heinold 2005; Etnier et al. 2006); and Virginia's 361 species (Parker and Voshell 1981; Flint et al. 2004; Flint et al. 2008; Flint et al. 2009). Based on the larger faunas reported from these states, the lack of distributional records from some river basins such as the Green River, and our overall fragmentary knowledge of the group within Kentucky, we concluded that additional survey efforts were warranted.

METHODS

Study area. Kentucky encompasses over 104,000 km² and spans a west to east distance of approximately 676 km from the Mississippi River in the west to its eastern border in Pike County (Burr and Warren 1986). It is bordered on the north and east by the Ohio River and Big Sandy Rivers, to the south by the state of Tennessee, and to the southeast by the Commonwealth of Virginia. Elevations range from a low of 79 m along the Mississippi River in Fulton County to a high of 1265 m on the summit of Black Mountain in Harlan County (Burr and Warren 1986).

Recognition of Kentucky's diverse geology, topography, and natural communities has resulted in the identification of numerous physiographic and natural regions for the Commonwealth (Fenneman 1938; Quartermann and Powell 1978; Burr and Warren 1986; Ulack et al. 1998; USEPA 2002; Woods

et al. 2002; Taylor and Schuster 2004; Abernathy et al. 2010). In the following discussion, we follow the classification scheme of USEPA (2002) to describe the diversity of Kentucky's distinct physiographic and natural regions; however, we also identify and incorporate several distinct subregions (Burr and Warren 1986; Ulack et al. 1998; Abernathy et al. 2010) based on their unique geology, topography, and biological characteristics (Figure 1).

The eastern one-third of Kentucky is comprised of the Central Appalachians, Western Alleghany Plateau, and Southwestern Appalachians ecoregions, a region collectively referred to as the "Eastern Coalfield." This mountainous region is characterized by deeply eroded plateaus, steep hills, sharp ridges, and narrow stream valleys in the north and west (i.e., Red River Gorge Geological Area and Big South Fork National River and Recreation Area) and by uplifted mountains and ridges in the southeast. The southeastern corner of the region contains the highest elevations in the state, specifically Pine, Cumberland, and Black mountains. The entire Eastern Coalfield region is underlain by Pennsylvanian-age sandstones, shale, and some coal-bearing deposits. Kentucky's two most significant areas of old-growth forest, Lilley Cornett Woods (Letcher County) and Blanton Forest State Nature Preserve (Harlan County), occur here. Four major rivers (Big Sandy, Licking, Kentucky, and Cumberland) originate within the region. Smaller streams of the region are typically cool with moderate to high-gradients, cobble or boulder substrates, and low nutrient and ionic concentrations. Activities associated with natural resource extraction (surface coal mining and logging) have altered water quality and physical habitats of many stream systems in the Eastern Coalfield (Abernathy et al. 2010).

The Interior Plateau is a diverse ecoregion composed of a series of plateaus, basins, and domes, often separated by distinct escarpments. The north-central portion of the region, commonly referred to as the "Bluegrass," is a broad upland area that developed on Ordovician-age limestone and shale. The topography of the Bluegrass varies from gently rolling uplands in the interior to more pronounced, steep-sided hills at the periphery. The region is crossed by three major rivers: the Kentucky, Licking, and Salt. The

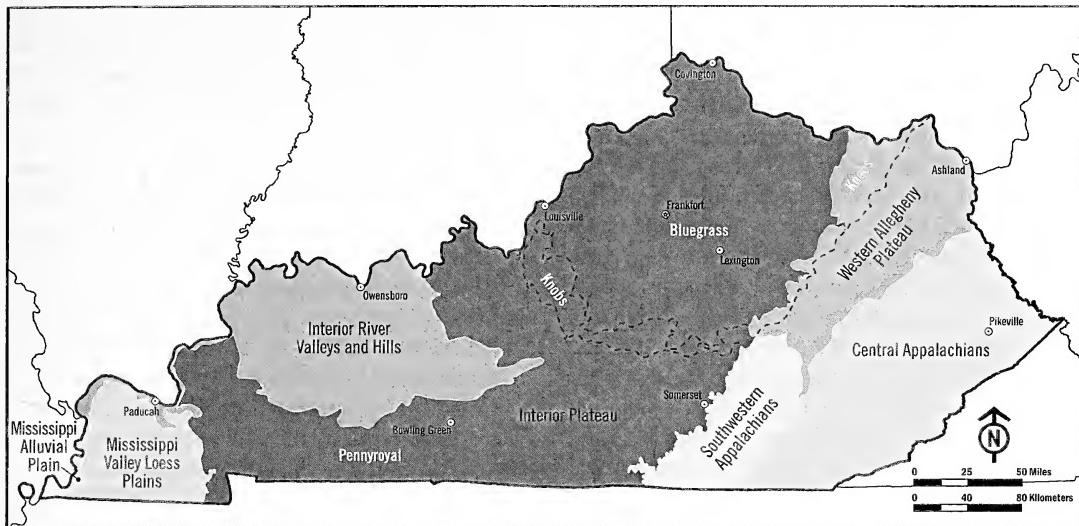


Figure 1. USEPA Level III Ecoregions of Kentucky (select subregions are labeled in white; the Knobs subregion is delineated with dashed lines) (USEPA 2002).

Kentucky River has carved a deep, sinuous gorge through the inner part of the region (the “Palisades”), exposing Kentucky’s oldest rock strata along the edges of the valley. Streams cutting through the Palisades region have steep gradients and typically exhibit greater insect diversity than other Bluegrass streams. Upland streams in the Bluegrass can be intermittent or perennial but tend to have low to moderate gradients, coarse substrates, and relatively high nutrient and ionic concentrations.

The Bluegrass is bordered on the west, south, and east by a series of well-defined, cone-shaped hills and ridge systems referred to as the “Knobs.” This narrow, semicircular band of hills is characterized by high gradient headwater streams and broad, often wet valleys. The Knobs are underlain primarily by Mississippian and Devonian-age shales, siltstones, and sandstones. The remainder of the Interior Plateau, often referred to as the “Pennyroyal” region, consists of a physiographically diverse, horseshoe-shaped area that extends from the Knobs to the western edge of the Tennessee River basin. The central portion of this region includes the well-known Mammoth Cave system, an area characterized by resistant, Mississippian-age sandstone ridges and bluffs overlying deep limestones in which extensive cave systems have developed. The balance of the region is less rugged, consisting of an extensive limestone upland

characterized by karst topography and the development of subterranean drainage through caverns and sinkhole plains. Two interesting aquatic habitats, springs and sinkhole ponds, occur here, and often support diverse and unique aquatic communities. The biologically diverse Green River originates within this region, as do many of its tributaries (i.e., Barren, Little Barren, Nolin, and Pond). Smaller streams in this region are highly variable with respect to gradient and substrate, but nutrient and ionic concentrations tend to be higher than in the Eastern Coalfields.

The Interior River Valleys and Hills, or “Western Coalfield,” is composed of hills and aggraded lowlands developed primarily on Pennsylvanian-age (coal-bearing) sandstones. The hills range from rugged, steep cliffs adjacent to streams and rivers to lower hills developed on shale or buried under Pleistocene-age loess deposits. The aggraded lowlands extend along the mainstem and tributaries of the Tradewater, Green, and Ohio rivers, and these lowlands contain some of Kentucky’s largest and most numerous oxbows, sloughs, and wetlands. Upland streams tend to have rocky substrates, while lowland streams are typically dominated by silt and sand substrates. Extensive surface and underground coal mining activities in the region have altered the water quality and habitat conditions of many streams.

The Mississippi Valley Loess Plains and Mississippi Alluvial Plain ecoregions in extreme western Kentucky are known collectively as the "Purchase" or "Jackson Purchase" region. The Mississippi Valley Loess Plains consists of a flat to rolling upland covered by windblown silt (loess) deposits and underlain by unconsolidated sediments that are susceptible to erosion. Bluff hills are common along its western edge near the Mississippi River. Agriculture is the typical land cover in the region, and the majority of major streams have been channelized. Streams in this ecoregion are low gradient with sand and gravel substrates. The Mississippi Alluvial Plain extends along the bottomland of the Mississippi River. It is mostly a broad, flat alluvial plain with river terraces, swales, and levees providing the main elements of relief. Unique lentic habitats such as abandoned channels, oxbow lakes, and cypress wetlands occur here. Bottomland deciduous forests covered the region before much of it was cleared for cultivation. Presently, most of the region is in agricultural production and most streams have been channelized. Streams in this ecoregion are low-gradient with sand and silt substrates.

Literature review and site selection. We compiled a baseline list of Kentucky caddisflies through reviews of all the published literature containing Kentucky records, reviews of several unpublished student theses, and an extensive search of the Kentucky Division of Water's (KDOW) macroinvertebrate database (Ecological Data Application System). Over the past 25 years, KDOW biologists have sampled in all of Kentucky's counties and major watersheds, producing thousands of taxa records. The majority of KDOW's collections were based on larvae, so species identifications could not be made for those families lacking larval keys (e.g., Hydroptilidae). Based on distributional information obtained from all of these sources, we focused our sampling efforts in areas not previously surveyed or in areas with unique natural or biological features (e.g., outstanding state resource waters [OSRW], state nature preserves [SNP], natural wetlands, sinkhole ponds, and springs). Over a seven-year period (2005–2011), we visited 150 sites across the Commonwealth, and approximately 20% of these sites were sampled on more than one

occasion in an attempt to cover the flight periods of as many species as possible. The majority of sampling efforts took place in the spring (April to June) or fall (September and October).

Field and laboratory methods. Our sampling efforts were focused on adult caddisflies, but a limited number of sites were searched for larvae. Adult caddisflies were collected with ultraviolet light traps, Malaise traps, and sweep netting of riparian vegetation, but the majority of collections were made using two different light trap devices: a bucket trap consisting of a 15-watt, black light positioned vertically over a bucket, funnel, and alcohol-filled jar, and a pan trap consisting of a 15-watt, black light (BioQuip, 12-volt, DC) positioned over top of an ethanol-filled white tray. The bucket trap was equipped with a digital timer that allowed us to set the trap at any time of day and retrieve it the following day. Both traps were powered by small, portable, 12-volt batteries. Sampling was conducted at dusk, beginning about 30 minutes prior to sunset and lasting for approximately 1–2 h. Adult caddisflies and other aquatic insects were attracted to the light, fell into the alcohol, and were preserved for future study. Larval caddisflies were collected with sweep nets, kick nets, or by hand-picking from rocks or other substrates. All collected organisms were placed in labeled jars and returned to the laboratory for sorting and identification. Caddisflies were sorted and identified to species level when possible (some female specimens were left at genus); remaining aquatic insects (e.g., stoneflies) were placed in ethanol-filled vials for future study. The abdomen of some specimens (whole body for Hydroptilidae) was removed and cleared in 10% KOH prior to examination.

Data analyses. Previous and current collection records were organized by family and combined to produce a new annotated checklist for the Commonwealth. The new checklist was evaluated to determine the total species richness, the number of new records, the species richness for each ecoregion, and other general distributional patterns. Imperiled or vulnerable taxa were identified using the natural heritage methodology employed by NatureServe (2012) and KSNPC (2011). Species were ranked based on the number of known occurrences (populations), the

number of individuals, the severity of potential threats, and other sources (Morse et al. 1997). Species with Global (G) and State (S) ranks of G1/S1 (critically imperiled), G2/S2 (imperiled), and G3/S3 (vulnerable) were identified from the checklist based on these factors. A “Q” qualifier at the end of a global rank indicates questions about the taxonomy of the species. Species were state-designated as Endangered (E), Threatened (T), or Special Concern (S) based on state rankings and our evaluations of potential threats.

Disposition of specimens. Vouchers for the majority of newly examined material have been deposited in the Branson Museum of Zoology at Eastern Kentucky University or the senior author’s personal collection. Specimens collected from U.S. National Parks (e.g., Cumberland Gap National Historical Park) have been deposited in the collection at Mammoth Cave National Park. Vouchers of new or unpublished distributional records have been deposited in the National Museum of Natural History.

RESULTS

In this paper, we present distributional records for 293 caddisfly species, including 22 families, 68 genera, and 69 new Kentucky records (see annotated list below). The family Hydroptilidae is represented by the most species (66), followed by the Hydropsychidae (45), Leptoceridae (44), Polycentropodidae (27), Limnephilidae (19), Rhyacophilidae (15), Glossosomatidae (12), and Thremmatidae (10). All other families are represented by fewer than 10 species. The family Sericostomatidae is reported from Kentucky for the first time. Among genera, *Hydroptila* is represented by the most species (31), followed by *Ceraclea* (16), *Cheumatopsyche* (16), *Polycentropus* (16), *Hydropsyche* (15), and *Rhyacophila* (15).

The mountainous region of eastern Kentucky (Eastern Coalfield) is represented by the greatest number of species (228) and also the greatest number of unique records (70). Among ecoregions, the most species have been recorded from the Interior Plateau (215), followed by the Central Appalachians (183), Southwestern Appalachians (176), Western Allegheny Plateau (110), Mississippi Valley Loess Plains (65), Interior River Valleys

and Hills (43), and Mississippi Alluvial Plain (34). Species’ distributions vary in scope, with some species occurring statewide (e.g., *Cheumatopsyche analis* (Banks), *Hydroptila consimilis* Morton, *Oecetis inconspicua* (Walker), and *Rhyacophila lobifera* Betten) and other species being limited to only one or two sites (e.g., *Brachycentrus lateralis* (Say); *Hydroptila keuhnei* Houp, Houp, and Harris; *Fumonta major* (Banks); and *Rhyacophila appalachia* Morse and Ross).

The following checklist is arranged alphabetically by family, genus, and species. Nomenclature generally follows that of Morse (2011). We follow Olah and Johnson (2008) and Geraci et al. (2010) with regard to the placement of *Ceratopsyche* and *Hydropsyche* and Vshivkova et al. (2007) with regard to the placement of *Neophylax*. General notes on taxonomy, biology, and distribution are provided for families and genera. Larvae of all Kentucky genera are keyed and illustrated in Wiggins (1996) and Merritt et al. (2008); however, family placement has changed for some genera. Pupae for most genera can be identified in keys provided by Ross (1944). Adults for all Kentucky families can be identified in keys provided by Merritt et al. (2008); Schmid (1980) and Ross (1944) provide adult keys for most Kentucky genera and some species.

The checklist is a compilation of literature records, KDOW specimens, records from unpublished reports, and our recent collections. Unpublished or new Kentucky records are marked with an asterisk (*), and imperiled or vulnerable species (species of conservation interest) are marked with a plus symbol (+). All records are based on adults unless otherwise noted. Kentucky distributions are described as statewide, a specific ecoregion or subregion (Figure 1), or a specific locality if the species’ distribution is limited to only a few sites (e.g., stream and county). Locality information is followed by adult flight period and the initial publication date (e.g., Resh 1975) for those species previously reported from Kentucky. For species with limited Kentucky distributions, the global range is summarized. Unless otherwise referenced, global distributions are based on Etnier et al. (1998), Flint et al. (2004, 2006, 2008), Morse (2012), and NatureServe (2012). For

imperiled or vulnerable species, we provide the global (G) (NatureServe 2012) and state (S) conservation ranks (KSNPC 2011), the state status (Threatened [T], Special Concern [S], or Historic [H] - not observed for at least 20 years and possibly extirpated) (KSNPC 2010), where applicable, and any other published designation or status (Morse et al. 1997). For species we believe to be imperiled or vulnerable, but without formally assigned G or S ranks or a state status, we have proposed ranks and statuses based on our own knowledge of the species' global and state range and status. Additional commentary is provided for rare species, new Kentucky records, species with limited Kentucky records, or those species which constitute significant range extensions.

Standard abbreviations are used for states and provinces (e.g., KY, TN, and WV). Other abbreviations are used as follows: Abraham Lincoln Birthplace National Historic Site (ABLI), Big South Fork National River and Recreation Area (BISO), Blue Grass Army Depot (BGAD), Branch (Br), County (Co), Creek (Crk), Cumberland Gap National Historical Park (CUGA), Daniel Boone National Forest (DBNF), Fork (Frk), Fort Campbell Military Reservation (Fort Campbell), Fort Knox Military Reservation (Fort Knox), Land Between the Lakes National Recreation Area (LBL), Kentucky Department of Fish and Wildlife Resources (KDFWR), Kentucky State Nature Preserves Commission (KSNPC), Mammoth Cave National Park (MACA), Outstanding State Resource Water (OSRW), State Nature Preserve (SNP), tributary (trib), United States (US), and wildlife management area (WMA).

Annotated Checklist

Family Apataniidae

Formerly a member of the Family Limnephilidae (Wiggins 1996; Gall 1997), the family Apataniidae contains 5 North American genera and 17 species (Wiggins 1996; Flint et al. 2008). A single genus, *Manophylax*, occurs in KY. *Manophylax* larvae generally occupy hygroscopic habitats (a thin film of water flowing over inclined rock surfaces in small spring seeps), and the adults are poor fliers (Wiggins 1996; Schuster 1997).

Genus *Manophylax*. The single Kentucky species, *M. butleri* Schuster, has a four-year life cycle, six larval instars, and the larvae are restricted to vertical sandstone rock faces that are wet for only a portion of the year. During dry periods, the larvae place a silk closure across the front of the case and go into a period of quiescence. The larvae remain inactive until rain causes the walls to become wet and the rock walls stay wet for some time. The adults are poor fliers and tend to remain on the walls from which they emerge. Its sister species, *M. altus* (Huryn and Wallace), is restricted to high elevation sites in three NC counties. Larval, pupal, and adult characters for *M. butleri* are provided in Schuster (1997) and Jones (2000).

+*Manophylax butleri* Schuster. Shillalah Crk WMA, Bell Co; DBNF, Carter Co; Cumberland Falls State Resort Park and DBNF, McCreary Co; Red River Gorge (DBNF), Wolfe Co; March–April (Schuster 1997). Range: KY, TN, and WV. Rank: G2, S2. KY Status: S. During this study, we discovered unknown populations at Shillalah Crk WMA (KDFWR), adjacent to CUGA in Bell Co and on rock outcrops overlooking Big South Frk Cumberland River (BISO) in Scott Co, TN near the KY/TN border. The Tennessee collection represents a new distributional record for that state. Our new records expand the species' range to the south and southeast; however, *M. butleri* remains vulnerable to extirpation due to its localized distribution and poor dispersal ability.

Family Brachycentridae

The Family Brachycentridae contains five North American genera, two of which occur in Kentucky: *Brachycentrus* and *Micrasema*. The larvae occur in lotic habitats and construct cases composed primarily of plant material.

Genus *Brachycentrus*. Three of the 14 North American species of *Brachycentrus* occur in KY. The larvae occupy medium-sized streams to large rivers, where they attach their cases to rocks, stationary wood, or aquatic vegetation. Keys, descriptions, and distributional information for larvae and adults of eastern species are provided in Flint (1984).

Brachycentrus lateralis (Say). Ohio River, Jefferson Co; May (Resh 1975). Range: widely distributed in eastern U.S. and Canada (ON and QC) but only one KY locality.

+*Brachycentrus nigrosoma* (Banks). Yellow Crk, Bell Co (larvae); Buck Crk, Pulaski Co; Rockcastle River, Laurel Co (larvae); April–May (Floyd and Schuster 1990). Range: widely distributed in eastern U.S. (AL, GA, KY, MD, ME, NC, NY, PA, SC, TN, VA, and WV). Rank: G5. Proposed KY rank and status: S2S3, S. We observed thousands of larvae attached to boulders in the Rockcastle River, but adult records are limited to one KY site.

Brachycentrus numerosus (Say). Scattered localities statewide except Western Coalfield (larger creeks and rivers); May (Resh 1975).

Genus *Micrasema*. Five of the 18 North American species of *Micrasema* occur in KY. The larvae occupy small streams, where they are usually found in clumps of aquatic mosses (Wiggins 1996). Larvae and adults of KY species can be identified using Chapin (1978).

Micrasema bennetti Ross. Dry Br (MACA), Edmonson Co; Steeles Run, Fayette Co; March–April (Resh 1975). Range: GA, KY, NC, SC, TN, VA, and WV. According to Chapin (1978), the lack of records for this species stems from its brief emergence period and diurnal behavior.

Micrasema charonis Banks. Widely distributed in Interior Plateau, Southwestern Appalachians, and Western Allegheny Plateau; April–May (Houp 1999).

Micrasema rusticum (Hagen). Trammel Frk, Allen Co; Little Yellow Crk, Bell Co; Hickman Crk, Jessamine Co; Gasper River, Logan Co; Cumberland River and Cogur Frk, McCreary Co; Buck Crk, Pulaski Co; Horselick Crk, Rockcastle Co; Little South Frk, Wayne Co; April–May (Resh 1975).

+**Micrasema scotti* Ross. Dry Frk, Metcalfe Co.; October. Range: AL, IN, KY, NC, TN, VA, and WV (Chapin 1978). Rank: G3G4. Proposed KY rank and status: S1S2, T. We discovered the new Kentucky population in Dry Fork, a small, spring-fed tributary of South Fork Little Barren River in Metcalfe County.

Micrasema wataga Ross. Sinking Crk, Laurel Co; Red River and Sinking Crk, Logan Co; Cumberland River, McCreary Co; Big South Fork Cumberland River (BISO); April–July (Resh 1975).

Family Calamoceratidae

The Family Calamoceratidae is represented by three genera and five species in North

America. Two genera, *Anisocentropus* and *Heteroplectron*, are known from KY.

Genus *Anisocentropus*. The only North American species, *Anisocentropus pyraloides* (Walker), is reported from KY for the first time. The flat, oval, leaf-like case is unique among North American caddisfly larvae. The adults were illustrated by Betten and Mosely (1940); the larva was illustrated by Wiggins (1996).

**Anisocentropus pyraloides* (Walker). Small DBNF streams in Southwestern Appalachians and several spring habitats in Pennyroyal (MACA); Edmonson, Jackson, Laurel, McCreary, Pulaski, and Whitley counties (adults and larvae); April, June, August. Range: widely distributed from NJ to FL and west to MS. This species was first collected in Kentucky by KDOW personnel and Johansen (2000), but these records were not published. We have recent material (2005–2007) from the DBNF (McCreary and Pulaski counties) and MACA (Edmonson Co).

Genus *Heteroplectron*. The only species in eastern North America, *Heteroplectron americanum*, was illustrated by Schmid (1983). Wiggins (1996) illustrated the larva. The larval case is unique among eastern caddisflies, consisting of a hollowed-out twig.

Heteroplectron americanum (Walker). Eagle Crk and Rock Crk, McCreary Co.; Cane Crk, Jackie Br, and South Frk Dogslaughter Crk, Whitley Co. (adults and larvae); July. Etnier et al. (1998) reported an emergence period of April–May for TN. This species is widely distributed in eastern North America (AL to QC).

Family Dipseudopsidae

The Family Dipseudopsidae is represented by one genus (*Phylocentropus*) in North America. Three species of *Phylocentropus* are known from KY. The larvae of *Phylocentropus* are retreat-makers, constructing buried, silken tubes covered by sand grains (Wiggins 1996).

Genus *Phylocentropus*. Larval keys for all KY species were provided by Sturkie and Morse (1998). Adults can be identified in Schuster and Hamilton (1984).

Phylocentropus carolinus Carpenter. Scattered localities in Central and Southwestern Appalachians, Pennyroyal, Western Coalfield, and Jackson Purchase; Breathitt, Christian, Edmonson, Graves, Hardin, Harlan, Laurel, McCreary, and Pulaski counties (adults and larvae); May–August (Resh 1975).

Phylocentropus lucidus (Hagen). Central Appalachians, Southwestern Appalachians, and Western Allegheny Plateau: Davis Br (CUGA), Bell Co; unnamed bog (MACA), Edmonson Co; Watts Crk (Blanton Forest SNP), Harlan Co; Big Dog Br (DBNF), Laurel Co; Bad Br (Bad Br SNP), Letcher Co; unnamed trib Dog Frk, Wolfe Co; June–August (Schuster and Hamilton 1984).

Phylocentropus placidus (Banks). Scattered localities in Eastern Coalfield, western Pennyroyal, and Jackson Purchase: Calloway, Crittenden, Elliott, Graves, Hickman, Marshall, Morgan, Rockcastle, and Whitley counties (adults and larvae); April–May, August (Resh 1975).

Family Glossosomatidae

The Family Glossosomatidae is represented by six genera in North America; four genera are known from KY. When present, members of this family are often one of the more conspicuous groups of caddisflies as the larvae graze on algae, diatoms, and fine organic particles on the uppermost, exposed surfaces of rocks. The larvae construct and are concealed within dome-like, rock cases that resemble a tortoise shell (Wiggins 1996).

Genus *Agapetus*. Larvae cannot be identified to species at the present time; Etnier et al. (2010) provided a taxonomic review of the eastern species, with descriptions of 12 new species. Six *Agapetus* species are known from KY, including *A. kirchneri*, one of the new species described in Etnier et al. (2010). *Agapetus* larvae are generally found in small, spring-fed streams.

Agapetus avitus Edwards. Southwestern Appalachians and Pennyroyal: Clinton, McCreary, Trigg, Warren, and Wayne counties; April–June (Etnier et al. 2006).

Agapetus hessi Leonard and Leonard. Southwestern Appalachians and Pennyroyal: Logan, Pulaski, Warren, and Wayne counties; April–May (Resh 1975).

Agapetus illini Ross. Southwestern Appalachians and Interior Plateau: Bullitt, Christian, Clinton, Fayette, Jackson, Jessamine, Lincoln, Logan, Mercer, Pulaski, Wayne, Whitley, and Woodford counties (adults and larvae); April–July (Resh 1975).

+*Agapetus kirchneri* Parker, Etnier, and Baxter. Davis Br (CUGA), Bell Co; (larvae); April–July (Etnier et al. 2010). Range: KY,

TN, and VA. Proposed rank: G2. Proposed KY rank and status: S1, T. Type locality is Station Creek (CUGA), Lee Co, VA, just southeast of the KY border.

Agapetus minutus Sibley. Robinson Forest, Breathitt Co; Blue Spring, Good Spring, and unnamed bog (all MACA), Edmonson Co; Kinniconick Crk, Lewis Co; April, June–August (Phillippi and Schuster 1987).

Agapetus tomus Ross. Davis Br (CUGA), Bell Co; Robinson Forest, Breathitt Co; Green Co. (no location provided); Sinking Crk, Laurel Co; Bad Br (Bad Br SNP), Colliers Crk, Poor Frk, and Smith Crk, Letcher Co; Big South Frk Cumberland River (BISO), Cogur Frk, and Eagle Crk (DNBF), McCreary Co; April–July (Resh 1975).

Genus *Glossosoma*. All but 3 of the 22 North American *Glossosoma* are restricted to mountainous regions of the western U.S. and Canada. Two species, *G. intermedium* and *G. nigrior*, have been recorded from springs or spring-fed streams in KY. No keys are available for larvae of *Glossosoma*; adult males were illustrated by Ross (1944) and Schmid (1982).

Glossosoma intermedium (Klapalek). Scattered localities statewide except Bluegrass and Jackson Purchase; March–April (Resh 1975).

Glossosoma nigrior Banks. Scattered localities statewide except Bluegrass and Jackson Purchase; April–October (Resh 1975).

Genus *Matrioptila*. This monotypic genus has been recorded from PA south to AL. The larva of *M. jeanae* is illustrated in Wiggins (1996); male genitalia were illustrated by Ross (1938).

Matrioptila jeanae (Ross). Cumberland River, Bell Co; Little Barren River, Green Co; Big Dog Br, Laurel Co; Whippoorwill Crk, Logan Co; Big South Frk Cumberland River (BISO), McCreary Co; Cumberland River, McCreary Co; Buck Crk system, Pulaski Co; Little South Frk, Wayne Co; April–July (Resh 1975).

Genus *Protoptila*. The genus is represented by 13 species in North America; three species have been recorded from KY. Larvae of *Protoptila* occupy warmer habitats than other glossosomatids. No keys are available for larvae; Ross (1941, 1944) provided adult illustrations of genitalia for the three KY species.

+*Protoptila alexanderi* Ross. Sturgis, Union Co; August (Resh 1975). Range: widely

distributed in TX and Mexico (Houghton and Stewart 1998, Baumgardner and Bowles 2005) and reported from one Kentucky locality by Resh (1975). Rank: G5. Proposed KY rank and status: S1S2, T. We have not been able to re-examine and confirm the disjunct Kentucky record reported by Resh (1975); consequently, we consider this record to be tentative.

Protoptila maculata (Hagen). Anderson, Bell, Franklin, Harrison, McCreary, Pulaski, Shelby, Spencer, Warren, and Whitley counties; May–September (Resh 1975).

Protoptila palina Ross. Davis Br (CUGA) and Cumberland River, Bell Co; Green River, Green Co and Hart Co; Sinking Crk, Laurel Co; Salt Lick Crk, Marion Co; May–September (Resh 1975).

Family Goeridae

This family is represented by four North American genera, two of which occur in KY. Within KY, the larvae are restricted to cool streams of the Eastern Coalfields and springs or spring-fed streams of the Interior Plateau (Pennyroyal).

Genus *Goera*. Flint (1960) provided larval keys, and Schmid (1983) provided illustrations of adult genitalia for KY species.

Goera calcarata Banks. Eastern Coalfield and southeastern Pennyroyal: Bell, Breathitt, Clinton, Laurel, Letcher, McCreary, Pulaski, and Wayne counties (adults and larvae); May–September (Resh 1975).

+**Goera fuscula* Banks. A single Kentucky locality: Good Spring (MACA), Edmonson Co; June, August. Range: GA, KY, MA, ME, NC, NY, PA, QC, SC, VT, and VA. Rank: G5. Proposed KY rank and status: S1S2, S. The discovery of this species in KY represents a northwestern range extension for the species, as the closest known records are from TN and VA.

Goera stylata Ross. Eastern Coalfield and springs or spring-fed streams of Pennyroyal: Davis Br (CUGA), Bell Co; Clemons Frk and Coles Frk (Robinson Forest), Breathitt and Knott counties; Poplar Spring (Fort Knox), Hardin Co; unnamed springs along Houchins Ferry Road and Good Spring (MACA), Edmonson Co; Buffalo Spring, Doe Run, and McCracken Spring, Meade Co; Dry Frk system, Metcalfe Co; unnamed spring-fed trib Lynn Camp Crk, Hart Co; Sinking Crk system, Laurel Co; Cumberland River,

McCreary Co (adults and larvae); May–August (Resh 1975).

Genus *Goerita*. Parker (1998) provided keys to larvae and adults.

Goerita betteni Ross. Headwater streams and spring seeps in Eastern Coalfield (Breathitt, Harlan, Letcher, Menifee, McCreary, and Whitley counties) and Pennyroyal (Edmonson, Green, and Taylor counties); June–July (Phillippi and Schuster 1987). Parker (1998) described the larval habitat of *G. betteni* as vertical or steep rock faces in thin films of water along forested ravines. We collected larvae from similar habitats in KY. Prior to our collections in 2005 and 2006, KY records were limited to sandstone-dominated habitats in the Eastern Coalfield (Phillippi and Schuster 1987; Jones 2000; Pond 2000); however, we discovered additional populations in the karst, limestone-dominated region of the Pennyroyal.

Family Helicopsychidae

Within North America, the family is represented by a single genus, *Helicopsyche*.

Genus *Helicopsyche*. The genus is represented by five species in North America north of Mexico. A single, wide-ranging species, *Helicopsyche borealis* (Hagen), occurs in KY. Its snail-like, helical case is unique among North American caddisflies and serves as a valuable diagnostic character in the field. Adults and larvae of *H. borealis* are described by Ross (1944) and Moulton and Stewart (1996).

Helicopsyche borealis (Hagen). Widespread and locally abundant in Interior Plateau, but less abundant in Eastern Coalfield and apparently absent from Western Coalfield and Jackson Purchase; May–October (Resh 1975). Zhou et al. (2011) provided evidence that *H. borealis* is a species complex composed of many, genetically distinct lineages exhibiting similar (if not identical) male genitalia.

Family Hydropsychidae

This is a large and dominant family represented by 10 genera in North America. The larvae are restricted to lotic habitats or wave-washed shorelines of lakes where they construct fixed, silken retreats to capture suspended food particles from the current. The family is extremely important to stream

ecology because of its wide-spread occurrence, abundance, and large biomass (Wiggins 1996).

Genus *Cheumatopsyche*. Within North America, the genus includes at least 50 species, many of which are very common and widely distributed. Several species are tolerant of water quality and habitat perturbations and can be quite abundant under those conditions. No larval keys are available, but descriptions and illustrations of Kentucky males and females were provided by Gordon (1974).

Cheumatopsyche analis (Banks). Widespread and common statewide; April–October (Resh 1975). One of the most common and pollution-tolerant caddisfly species in eastern North America. It is often abundant at disturbed or impaired sites.

Cheumatopsyche aphanta Ross. Robinson Forest, Breathitt Co; Moore Crk, Knox Co; unnamed trib Dog Frk, Wolfe Co; June–July (Resh 1975).

Cheumatopsyche burksi Ross. John James Audubon State Park, Henderson Co; no specific locality, Oldham Co; Schoolhouse Hollow Spring and Turkey Spring (both LBL), Trigg Co; June, August, October (Resh 1975).

Cheumatopsyche campyla Ross. Statewide; May–September (Resh 1975).

Cheumatopsyche geora Denning. Davis Br and Tunnel Crk (both CUGA), Bell Co; Grindstone Crk, Calloway Co; Crooked Crk (LBL), Trigg Co; May–September (Resh 1975).

+**Cheumatopsyche gyra* Ross. Davis Br (CUGA), Bell Co; September. Range: east coast from ME to GA. Rank: G4G5. Proposed KY rank and status: S1S2, T. Its collection in KY represents a western range extension.

**Cheumatopsyche halima* Denning. Davis Br and Shillalah Crk, Bell Co.; Colliers Crk, Letcher Co; June. Range: eastern North America from QC to SC and west to AR.

Cheumatopsyche harwoodi harwoodi Denning. Scattered localities in Central Appalachians, plus isolated localities in Pennyroyal, Western Coalfield and Jackson Purchase: Bell, Edmonson, Graves, Harlan, Henderson, Knox, Laurel, Letcher, McCreary, and Pulaski counties; April–September (Resh 1975).

+*Cheumatopsyche helma* Ross. This species has been documented from only one Kentucky locality: Pineville, Bell Co; June (Resh 1975).

Range: AL, AR, KY, ME, NC, PA, TN, and WV. Rank: G3, SH; KY status: H. This species has not been observed in KY for over 30 years and is possibly extirpated (KSNPC 2010, 2011).

Cheumatopsyche minuscula (Banks). Eastern Coalfield: Cumberland River (Pineville), Bell Co; Watts Crk (Blanton Forest SNP), Harlan Co; Big South Frk Cumberland River (BISO), McCreary Co; Burnside, Pulaski Co; April–August (Resh 1975).

Cheumatopsyche oxa Ross. Widespread in Eastern Coalfield and eastern Pennyroyal; April–October (Resh 1975).

Cheumatopsyche pasella Ross. Scattered localities in Bell, Breathitt, Edmonson, Green, Graves, Hart, McCreary, and Spencer counties; May–September (Resh 1975).

**Cheumatopsyche pinaca* Ross. Blood River, Calloway Co; Terrapin Crk SNP, Graves Co; Beaver Crk, Cogur Frk, and Eagle Crk (all DBNF), McCreary Co; Big South Frk Cumberland River (BISO), McCreary Co; May–June. Range: widely distributed along eastern seaboard from ME to LA. Its collection in KY represents a western range extension.

Cheumatopsyche sordida (Hagen). Cumberland River (Pineville), Bell Co; Cumberland River, McCreary Co; Rockcastle River (Livingston), Rockcastle Co; May–June, August (Resh 1975).

Cheumatopsyche speciosa (Banks). Salt River, Spencer Co; July (Ross 1944). Range: widely distributed throughout eastern U.S. and Canada. Within KY, this species appears to be restricted to the Salt River system (Resh et al. 1975; Haag et al. 1984).

Genus *Diplectrona*. Five species of *Diplectrona* are known from North America north of Mexico, including one species from western North America. Larvae and adults of the two Kentucky species, *D. modesta* Banks and *D. metaqui* Ross, are described in Ross (1944, 1970) and Wiggins (1996).

Diplectrona metaqui Ross. Springs, spring seeps, or small, spring-fed streams in seven, widely separated counties: Bell, Bullitt, Edmonson, Franklin, Hardin, Harlan, Hart, Larue, McCreary, Powell, Taylor, and Trigg; all KY records based on larvae, flight period unknown (Phillippi and Schuster 1987). Larval habitats for this species are often overlooked by researchers and not typically sampled by water resource agencies; consequently, the species is

rarely observed and likely underrepresented in larval surveys. The flight period in TN was reported as April–early June (Etnier et al. 1998).

Diplectrona modesta Banks. Statewide in small, cool streams, but less common in western Pennyroyal and Jackson Purchase; April–October (Resh 1975). Zhou et al. (2011) provided evidence that *D. modesta* is a species complex composed of morphologically similar forms with substantial genetic divergence.

Genus *Homoplectra*. About 12 species are known from North America, with three species limited to the east (Flint et al. 2004). Larvae occur in intermittent streams and seeps (Wiggins 1996). Larval and adult descriptions were provided by Ross (1944) and Weaver et al. (1979).

Homoplectra doringa (Milne). Scattered localities in Eastern Coalfield and eastern half of Interior Plateau (adults and larvae); April–May, July (Resh 1975).

Genus *Hydropsyche*. Within North America, the genus is represented by about 76 species (Flint et al. 2004), approximately one-third of which (23) occur in KY. The taxonomy of the genus *Hydropsyche* has been controversial, with much disagreement over the placement of *Ceratopsyche* and *Hydropsyche* (Schuster and Etnier 1978; Schuster 1984; Scheftet et al. 1986; Morse 1993). We follow recent proposals by Olah and Johnson (2008), which synonymized *Ceratopsyche* with *Hydropsyche*, and Geraci et al. (2010), which established new species groups for the genus *Hydropsyche* – *H. bronta* Group (generally corresponds with *Ceratopsyche*, *H. (Ceratopsyche)*, *H. morosa* Group, or *H. newae* Group) and *H. instabilis* Group (generally corresponds with *Hydropsyche* s.s.). Larval descriptions of eastern species were provided by Schuster and Etnier (1978) and Scheftet and Wiggins (1986). Male and female descriptions for some Kentucky species were provided by Ross (1944).

Hydropsyche Bronta Group

**Hydropsyche alhedra* Ross. Central Appalachians: Dark Ridge Br, Davis Br, Martins Frk, and Shillalah Crk (all CUGA), Bell Co; July–September. Range: widely distributed from Alaska, across Canada and the northern US, with extensions along the Rocky Mountains to CO and the Appalachian Mountains to TN and NC.

Hydropsyche bronta Ross. Scattered localities in Eastern Coalfield: Bell, Breathitt, Clay, Estill, Floyd, Harlan, Jackson, Knott, Leslie, Letcher, Martin, Owsley, Perry, and Pike counties; June–August (Resh 1975).

Hydropsyche cheilonis Ross. Widespread in Eastern Coalfield and eastern half of Interior Plateau; May–September (Resh 1975).

+*Hydropsyche etnieri* (Schuster and Talak). Peter Br of Little Wolf Crk, Whitley Co; no flight period information provided (Etnier et al. 1998). We also have records from near the KY border at Gap Crk (CUGA), Lee Co, VA. Range: KY, TN, and VA. Rank: G2. Proposed KY rank and status: S1S2, T. Ranked as “Rare and Vulnerable” by Morse et al. (1997).

Hydropsyche morosa Hagen. Central Appalachians: Martins Frk, Shillalah Crk, and Sugar Run (all CUGA), Bell Co; Robinson Forest, Breathitt Co; March, June–September (Resh 1975).

Hydropsyche slossonae (Banks). Scattered localities in Pennyroyal and Eastern Coalfield: Allen, Barren, Bell, Christian, Green, Harlan, Hart, Jackson, Knott, Lee, Letcher, Martin, Meade, Metcalfe, Monroe, Pike, Trigg, and Wayne counties; May–June, August, October (Thoeny and Batch 1983).

Hydropsyche sparna Ross. Widespread and common statewide; April–September (Resh 1975).

Hydropsyche ventura Ross. Central and Southwestern Appalachians: Bell, Breathitt, Harlan, Jackson, Laurel, Letcher, and Pike counties; May–June, August (Phillippi and Schuster 1987).

Hydropsyche Instabilis Group

Hydropsyche aerata Ross. Mayfield Crk, Carlisle Co; Rough River, Hardin Co; July (Houpt 1999).

**Hydropsyche alvata* Denning. Green River, Edmonson Co; June. Range: AL, AR, FL, GA, IL, IN, KY, LA, MI, MO, MS, NC, OK, SC, and VA.

Hydropsyche betteni Ross. Statewide; April–October (Resh 1975). This species is very common and appears to be one of Kentucky’s most pollution-tolerant caddisflies, based on its abundance at impaired sites.

Hydropsyche bidens Ross. Mayfield Crk, Carlisle Co.; Green River, Green Co; July (Houpt 1999).

Hydropsyche cuanis Ross. Pennyroyal: Russell Crk, Adair Co; Green River, Green Co; Red River, Logan Co; June–July (Houp 1999).

Hydropsyche depravata (Hagen). Scattered localities in Pennyroyal: Caldwell, Logan, Lyon, Meade, Metcalfe, Simpson, Warren, and Wayne counties and two localities in CUGA, Bell Co; April, June–July, September–October (Resh 1975).

Hydropsyche dicantha Ross. Statewide; April–August (Resh 1975).

Hydropsyche frisoni Ross. Eastern Coalfield and Pennyroyal; May–August (Thoeny and Batch 1983).

Hydropsyche hageni Banks. Cumberland River mainstem, Bell, McCreary, and Whitley counties; Bark Camp Crk, Whitley Co; April–June, August (Resh 1975).

+**Hydropsyche mississippiensis* Flint. Blood River, Calloway Co; April. Range: AL, FL, KY, LA, MS, NC, SC, TN, TX, and VA. Rank: G5. Proposed KY rank and status: S2S3, S. Our KY record represents a northern range extension for the species.

Hydropsyche orris Ross. Scattered localities in Western Allegheny Plateau, Interior Plateau, Western Coalfield, and Jackson Purchase; June–September (Resh 1975).

Hydropsyche patera Schuster and Etnier. Elkhorn Crk system, Franklin and Scott counties; June (Houp 1999).

Hydropsyche phalerata Hagen. Cumberland River mainstem, Bell, McCreary, and Whitley counties; unnamed bogs and Green River mainstem (MACA), Edmonson Co; April, June (Resh 1975).

Hydropsyche rossi Flint, Voshell, and Parker. East Frk Clarks River (Clarks River NWR), Marshall Co; Horselick Crk, Rockcastle Co; Salt River, Spencer Co; Barren River, Warren Co; May–July (Houp 1999).

Hydropsyche simulans Ross. Statewide; May–September (Resh 1975).

Hydropsyche valanis Ross. Scattered localities statewide, except Jackson Purchase; July (Resh 1975).

Hydropsyche venularis Banks. Cumberland River (Pineville), Bell Co; Big South Frk Cumberland River (BISO), McCreary Co; June (Resh 1975).

Genus *Macrosteleum*. The genus is represented by three eastern species, one of which, *Macrosteleum zebratum* Hagen, occurs

in KY. Among the North American hydropsychids, nets of *Macrosteleum* larvae have the smallest mesh size (5 × 40 microns) (Wallace and Sherberger 1974). The adults are distinctive based on their long antennae and brightly colored, yellow and black wings. Larval and adult descriptions of *M. zebratum* were provided by Ross (1944).

Macrosteleum zebratum Hagen. Large streams and rivers of eastern Pennyroyal, Southwestern Appalachians, and Central Appalachians: Adair, Bell, Casey, Green, Harlan, Laurel, Marion, McCreary, Owsley, and Pulaski counties (adults and larvae); May–September (Resh 1975).

Genus *Parapsyche*. Larvae of *Parapsyche* typically inhabit cold, running waters, where the retreats are located in strong currents (Wiggins 1996). Keys are provided in Flint (1961). Only one species, *Parapsyche cardis* Ross, is known from KY and is reported here for the first time.

**Parapsyche cardis* Ross. Central Appalachians: Shillalah Crk (CUGA), Bell Co; Watts Crk, Blanton Forest SNP, Harlan Co; Bad Br, (Bad Br SNP), Letcher Co (adults and larvae); May–June. Range: GA, KY, NC, SC, TN, and VA. We have EKU and KDOW records of *P. cardis* from the 1980s and 1990s, but the records were not published. We collected additional individuals from Bad Branch and Watts Creek in 2007 and from Shillalah Creek in 2010. All three streams have excellent water quality.

Genus *Potamyia*. Only one species, *P. flava* occurs in eastern North America. Descriptions of larvae are provided by Wiggins (1996); adults are illustrated in Ross (1944).

Potamyia flava (Hagen). Scattered localities in Western Allegheny Plateau, Interior Plateau, Western Coalfield, and Jackson Purchase (large streams and rivers); May–September (Resh 1975).

Family Hydroptilidae

Members of the Family Hydroptilidae (microcaddisflies) are the smallest caddisflies in North America. The family is represented by 16 genera and 225 species in North America north of Mexico, and it is the largest caddisfly family in KY, with 11 genera and 65 species. Unless otherwise noted below, diagnostic keys and descriptions are unavailable for the majority of larvae and females. Ross

(1944) and Bickle (1979) provide male descriptions and illustrations for the majority of Kentucky species.

Genus *Agraylea*. Ross (1944) described the final larval instar of the only Kentucky species, *A. multipunctata*. The larvae construct a silken case interspersed with algal filaments. Larval habitats include lakes, ponds, or slow flowing portions of rivers, where they feed on filamentous algae (Wiggins 1996).

Agraylea multipunctata Curtis. Doe Run, Meade Co; Three Springs (MACA), Edmonson Co (adults and larvae); April (Minckley 1963; Resh 1975). Range: Canada and northern US, with records extending to TN and VA.

Genus *Dibusa*. This monotypic genus is represented by one eastern species, *Dibusa angata* Ross. It is the largest hydroptilid in North America, with larvae reaching lengths up to 6.7 mm (Wiggins 1996). The life history of this species is unique among eastern caddisflies because the final larval instar feeds on the red alga, *Lemanea australis*, and incorporates the same algal material into its case (Resh and Houp 1986).

Dibusa angata Ross. Scattered localities in Eastern Coalfield, Bluegrass, and Knobs: Breathitt, Fleming, Jessamine, Johnson, Laurel, Lewis, Madison, McCreary, Pendleton, Pulaski, and Woodford counties; April–May (Resh 1975).

Genus *Hydroptila*. The genus contains more than 125 species in North America, with 31 species reported here for KY.

Hydroptila ajax Ross. Russell Crk, Adair Co; Troublesome Crk, Breathitt Co; Mitchell Crk and White Oak Crk (Sinking Crk basin), Laurel Co; Brashears Crk and Salt River, Spencer Co; May–September (Resh 1975).

Hydroptila amoena Ross. Scattered localities in Central Appalachians, Southwestern Appalachians, and Interior Plateau: Anderson, Bell, Breathitt, Bullitt, Hart, Laurel, Madison, McCreary, and Rockcastle counties; May–October (Resh 1975).

Hydroptila ampoda Ross. Unnamed trib Line Frk (Lilley Cornett Woods), Letcher Co; unnamed trib Dog Frk, Wolfe Co; June–July. Range: northeastern U.S. (CT, KY, ME, MN, NH, PA, and TN) and Canada (NB, NS, and QC). Rank: G5. Proposed KY rank and status: S1S3, S. This species was first collected in KY

by Jones (2000), but his record was not published. We collected additional specimens in 2010 from Big Everidge Hollow (Lilley Cornett Woods), Letcher Co.

Hydroptila angusta Ross. Salt River, Anderson Co; Salt River and Brashears Crk, Spencer Co; June–September (Resh 1975).

Hydroptila armata Ross. Scattered localities in Central Appalachians, Southwestern Appalachians, and Interior Plateau; April–October (Resh 1975).

Hydroptila consimilis Morton. Scattered localities in Central Appalachians, Southwestern Appalachians, Western Allegheny Plateau, Interior Plateau, and one locality in Jackson Purchase (Terrapin Crk SNP); May–October (Resh 1975).

+**Hydroptila coweetensis* Huryn. Coles Frk (Robinson Forest), Breathitt Co; unnamed spring and Three Springs area (both MACA), Edmonson Co; Powder Mill Crk (Sinking Crk basin), Laurel Co; August. Range: AL, KY, NC, and VA (Morse et al. 1997). Rank: G1G2. Proposed KY rank and status: S1S2, T. Johansen (2000) provided the first KY record for this species during his intensive survey of the Sinking Crk basin in Laurel Co. We obtained additional records in 2007 and 2008 from spring and small stream habitats at MACA and Robinson Forest, respectively. Ranked as “Rare and Vulnerable” by Morse et al. (1997).

+**Hydroptila decia* Etnier & Way. Big Dog Br (Sinking Crk basin), Laurel Co; May. Range: AL, KY, TN, and VA (Morse et al. 1997). Rank: G2. Proposed KY rank and status: S1S2, T. Johansen (2000) provided the first KY record for this species during his intensive survey of the Sinking Crk basin in Laurel Co. We also have records from CUGA, Lee Co, VA. Ranked as “Rare and Vulnerable” by Morse et al. (1997).

Hydroptila delineata Morton. Three Springs area (MACA), Edmonson Co; Salt Lick Crk, Marion Co; Big South Frk Cumberland River (BISO), McCreary Co; Cumberland River, Whitley Co; April, July–August (Resh 1975).

+**Hydroptila fiskei* Bickle. Sinking Crk and White Oak Crk, Laurel Co; July–August. Range: KY, ME, NC, NH, PA, TN, and VA. Rank: G4. Proposed KY rank and status: S1S3, T. Johansen (2000) provided the only KY records for this species during his intensive

survey of the Sinking Crk basin in Laurel Co, but his records were not published.

Hydroptila grandiosa Ross. Eastern Coalfield (Bell, Breathitt, Johnson, Laurel, Letcher, McCreary, Pulaski, and Wolfe counties) and one Bluegrass locality (Muddy Crk, Blue Grass Army Depot, Madison Co); May–September (Resh 1975).

Hydroptila gunda Milne. Scattered localities in Central Appalachians, Interior Plateau, and Jackson Purchase (Bell, Edmonson, Graves, Letcher, Logan, Meade, Pulaski, Simpson, and Trigg counties); May–July, October (Floyd and Schuster 1990).

Hydroptila hamata Morton. Central and Southwestern Appalachians (Bell, Breathitt, Johnson, Laurel, McCreary, and Pulaski counties), Bluegrass (Franklin, Mercer, and Spencer counties), and western Pennyroyal (Trigg Co); May–September (Resh 1975).

+*Hydroptila howelli* Houp, Houp, and Harris. One of two Kentucky endemics, *H. howelli* has been documented from three widely separated localities: Sinking Crk system, Laurel Co; Salt Lick Crk, Marion Co; unnamed spring seep (Red River Gorge, DBNF), Menifee Co; April–May, July (Houp et al. 1998). Rank: G2G3. Proposed KY rank and status: S1S2, T.

Hydroptila jackmanni Bickle. Unnamed trib Casey Crk and Dry Frk (Fort Campbell), Trigg Co; May–June (Etnier et al. 2006).

+*Hydroptila kuehnei* Houp, Houp, and Harris. Blue Spring and Good Spring (MACA), Edmonson Co; Salt Lick Crk, Marion Co; McCracken Spring, Meade Co; Dry Frk basin, Metcalfe Co; April–October (Houp et al. 1998). One of two Kentucky endemics, *H. kuehnei* is restricted to spring or spring-fed streams of the Pennyroyal. Rank: G1G2. Proposed KY rank and status: S1S2, T.

+*Hydroptila lennoxi* Bickle. Clemons Frk (Robinson Forest) and Frozen Crk, Breathitt Co; Big Dog Br and White Oak Crk (Sinking Crk basin), Laurel Co; unnamed trib Dog Frk, Wolfe Co; June–July. Range: AL, KY, NH, and VA. Rank: G2G4. Proposed KY rank and status: S1S3, T. Johansen (2000) and Jones (2000) provided the first Kentucky records for this species, but their records were not published.

+*Hydroptila oneili* Harris. Western Pennyroyal (Fort Campbell): unnamed trib Casey Crk, Trigg Co; Noahs Spring, Christian Co;

June (Etnier et al. 2006). Range: AL, AR, GA, KY, and TN. Rank: G2G3. Proposed KY rank and status: S1S2, S.

+**Hydroptila paramoena* Harris. Little Yellow Crk and Tunnel Crk (CUGA), Bell Co; Big South Frk Cumberland River (BISO), McCreary Co; July–August. Range: mountainous regions of AL, GA, KY, and TN. Rank: G2G3. Proposed KY rank and status: S1S2, S.

+**Hydroptila paraxella* Harris and Armitage. Muddy Crk (BGAD), Madison Co; Thompson Br, Simpson Co; April–May. Range: KY and OH. Proposed rank: G3. Proposed KY rank and status: S2S3, S. Our specimens were examined by Steve Harris (Clarion University, Pennsylvania), who identified them as a recently described species from Ohio (Armitage et al. 2011).

Hydroptila perdita Morton. Scattered localities in Central Appalachians, Southwestern Appalachians, and Interior Plateau; Anderson, Bell, Breathitt, Casey, Clark, Franklin, Green, Jessamine, Johnson, Madison, Meade, Pulaski, Spencer, and Woodford counties; May–October (Resh 1975).

**Hydroptila quinola* Ross. Jackson Purchase and Southwestern Appalachians (Sinking Crk basin): Blood River, Calloway Co; Terrapin Crk SNP, Graves Co; Big Dog Br and Powder Mill Br, Laurel Co; East Frk Clarks River, Marshall Co; April–August, October. Range: widely distributed in eastern U.S. and Canada.

**Hydroptila remita* Bickle and Morse. Little Yellow Crk (CUGA), Bell Co; Sloans Crossing Pond (MACA), Edmonson Co; April, July–September. Range: AL, AR, FL, KY, LA, ME, MS, NC, NH, NJ, PA, SC, TN, and TX.

+*Hydroptila sandersoni* Mathis and Bowles. Buck Crk system, Pulaski Co; May–August (Floyd and Schuster 1990). Range: AL, AR, KY, MO, OH, OK, and TN. Rank: G3G4. Proposed KY rank and status: S1S2, S.

+**Hydroptila scolops* Ross. Little Yellow Crk (CUGA), Bell Co; April, July, September. Range: IL, KS, KY, MB, MN, TX, and WI. Rank: G4. Proposed KY status and rank: S1S3, T. Our KY record represents a southeastern range extension for the species.

Hydroptila spatulata Morton. Scattered localities in Central Appalachians and Interior Plateau: Barren, Bell, Breathitt, Bullitt, Hart,

Wayne, and Whitley counties; May–August (Resh 1975).

Hydroptila talladega Harris. Coles Frk (Robinson Forest), Breathitt Co; Sinking Crk basin, Laurel Co; April–May, July–August (Houp 1999). Limited to two KY localities but entire range includes AL, GA, KY, NC, OH, PA, SC, and VA.

Hydroptila vala Ross. Robinson Forest, Breathitt Co; Good Spring (MACA), Edmonson Co; Sinking Crk basin, Laurel Co; Beaver Crk, Cogur Frk, and Eagle Crk (all DBNF), McCreary Co; Pitman Crk, Pulaski Co; unnamed trib Dog Frk, Wolfe Co; May–June (Resh 1975).

Hydroptila virgata Ross. Robinson Forest, Breathitt Co; Pitman Crk, Pulaski Co; May–June (Resh 1975).

Hydroptila waskesia Ross. Russell Crk, Adair Co; Trammel Frk, Allen Co; Pitman Crk, Pulaski Co; April–May (Houp 1999).

Hydroptila waubesiana Betten. Scattered localities statewide; May–October (Resh 1975).

Genus *Ithytrichia*. Ross (1944) described the larva of the only Kentucky species. Larvae are strongly compressed and are distinguished from other hydroptilid genera by the presence of prominent, lobate projections on the abdomen (Moulton et al. 1999). The larval case is transparent and made of silk (Wiggins 1996).

Ithytrichia mazon Ross. Salt River, Spencer Co; June (Resh 1975). Range: AR, IL, KY, OH, and OK.

Genus *Leucotrichia*. Flint (1970) provided diagnostic characters for larvae and adults of the only Kentucky species, *L. pictipes* (Banks). Final instar larvae construct flattened, elliptical cases that are fastened immovably to rocks (resembling the egg cases of leeches). Final instar larvae graze on diatoms by extending their bodies through the anterior opening of the case (Wiggins 1996).

Leucotrichia pictipes (Banks). Scattered localities in Eastern Coalfield and Interior Plateau: Bell, Edmonson, Fleming, Harlan, Mercer, Powell, and Pulaski counties (larvae); Houp (1999).

Genus *Mayatrichia*. Ross (1944) described the larva of the only Kentucky species. Larvae construct tapered, silken cases that are reinforced with longitudinal or circular silken ridges. The preferred larval habitat is rocks in rapid sections of rivers and large streams (Wiggins 1996).

Mayatrichia ayama Mosely. Cumberland River, Bell Co; Indian Crk, McCreary Co; Big South Frk Cumberland River (BISO), McCreary Co; April–July (Resh 1975).

Genus *Metricchia*. Within North America north of Mexico, the genus is known only from AZ, OK, and TX. The larvae construct a silken case with attached algal filaments.

**Metricchia* sp. The KDOW has collected larvae from four KY counties (Knox, Lewis, Magoffin, Morgan) that possess morphological characters typical of *Metricchia* (M. Vogel, KDOW, pers. comm., 10 November 2009). The specimens have long, sparse setae on the anterior margin of all three thoracic nota; stout, strongly curved tarsal claws; and lateral humps on abdominal segments II and IV. Adult specimens are needed for a positive identification, but it appears that these KY records represent a significant range extension for the genus.

Genus *Neotrichia*. The genus is represented by 16 species in North America north of Mexico. Five species are known from KY. These are the smallest caddisflies in North America, with a maximum length of about 2.5 mm (Wiggins 1996).

Neotrichia collata Morton. This species was first reported from Kentucky by Ross (1944), but no specific locality was given. We have recent collections from Sugar Run (CUGA), Bell Co; March, July. Range: limited to one KY locality but widely distributed in eastern U.S. (AL, IL, KY, ME, NY, SC, and VT), with one outlying record from UT.

Neotrichia minutissimella (Chambers). Rockcastle River (Livingston), Rockcastle Co; June (Resh 1975). Limited to one KY locality but widely distributed in central and eastern US.

Neotrichia okopa Ross. Salt River, Anderson Co; Brashears Crk and Salt River, Spencer Co; June (Resh 1975).

+*Neotrichia riegeli* Ross. Paint Crk, Johnson Co; June–August. June–August (Resh 1975). Range: AR, IL, KY, and OK. Rank: G3. Proposed KY rank and status: S1S3, S.

Neotrichia vibrans Ross. Troublesome Crk, Breathitt Co; East Frk Clarks River (Clarks River NWR), Marshall Co; Big South Frk Cumberland River (BISO), McCreary Co; and trib of Casey Crk, Trigg Co; April–May, July–September (Etnier et al. 2006).

Genus *Ochrotrichia*. About 50 species are known north of Mexico; 10 species are now

recorded from KY. Larvae occur in a variety of lotic habitats.

Ochrotrichia anisca (Ross). Clemons Frk (Robinson Forest), Breathitt Co; June (Resh 1975). Limited to one KY locality but entire range includes AR, IL, KS, KY, MO, OK, and TX.

Ochrotrichia arva (Ross). Unnamed spring trib Lynn Camp Crk, Hart Co; unnamed trib East Frk Clear Crk, Jessamine Co; Mill Springs, Wayne Co; Clear Crk and Lees Br, Woodford Co; April–June (Houp 1999).

Ochrotrichia confusa (Morton). Hickman Crk at Indian Falls, Jessamine Co; April (Resh 1975). Limited to one KY locality but entire range includes AL, FL, KY, NC, NY, OH, ON, PA, SC, and TN.

Ochrotrichia eliaga (Ross). Widespread in Interior Plateau: Adair, Allen, Barren, Bullitt, Edmonson, Fayette, Franklin, Hart, Logan, Madison, Pulaski, Simpson, Taylor, Warren, and Woodford counties; April–August (Houp 1999).

Ochrotrichia reisi Ross. Scattered localities in Bluegrass, Pennyroyal, and Eastern Coalfield: Breathitt, Edmonson, Fayette, Hart, Jessamine, Laurel, Madison, Mercer, Taylor, Wolfe, and Woodford counties; April–June, August (Houp 1999).

Ochrotrichia shawnee (Ross). Scattered localities in Bluegrass and Pennyroyal: Adair, Allen, Barren, Bullitt, Jessamine, Marion, and Taylor counties, plus the Buck Crk system (Pulaski Co) at the boundary of the Interior Plateau and Southwestern Appalachians; May–June (Resh 1975).

Ochrotrichia spinosa (Ross). Scattered localities in Bluegrass (Jessamine, Madison, Mercer, Spencer, and Woodford counties), plus one locality in Knobs (Salt Lick Crk, Marion Co); April–July (Resh 1975).

Ochrotrichia tarsalis (Hagen). Scattered localities in Bluegrass, Pennyroyal, and Eastern Coalfield: Adair, Bell, Breathitt, Edmonson, Green, Hart, Laurel, Spencer, and Whitley counties; May–October (Resh 1975).

+**Ochrotrichia wojcickyi* Bickle. Elk Lick Crk (Floracliff SNP), Fayette Co; June. Range: limited to one KY locality but entire range includes IN, KY, MB, ME, MN, NH, OH, PA, TN and VA. Rank: G4. Proposed KY rank and status: S1S3, S. Elk Lick Crk is a steep, cascading, bedrock/cobble stream of the Kentucky River Palisades.

Ochrotrichia xena (Ross). Scattered localities in Bluegrass and eastern Pennyroyal: Breckinridge, Madison, Spencer, and Woodford counties; May (Resh 1975).

Genus *Orthotrichia*. The genus is represented by six species in North America; three species are known from KY. The larvae occupy lentic habitats or slowly flowing sections of streams or rivers.

Orthotrichia aegerfasciella (Chambers). Statewide; May–September (Resh 1975).

Orthotrichia cristata Morton. Scattered localities statewide: Anderson, Bell, Bullitt, Edmonson, Graves, Henderson, Laurel, Letcher, Pulaski, and Spencer counties; May–August (Resh 1975).

+**Orthotrichia curta* (Kingsolver and Ross). Sinking Crk, Laurel Co; June–August. Range: AL, FL, KY, LA, ME, MN, NJ, QC, and TX. Rank: G4. Proposed KY status and rank: S1S2, T. Johansen (2000) provided the only KY records for this species during his intensive survey of the Sinking Crk basin in Laurel Co.

Genus *Oxyethira*. The genus is represented by about 40 species in North America north of Mexico; 8 species are now known from KY. Fifth-instar larvae construct a distinctively flattened, flask-shaped, clear case and occupy lentic habitats or slowly flowing portions of streams.

Oxyethira forcipata Moseley. Davis Br and Yellow Crk (both CUGA), Bell Co; Wilson Crk (Bernheim Forest), Bullitt Co; Buck Crk, Pulaski Co; White Oak Crk, Laurel Co; Hematite Lake (LBL), Trigg Co; April–October (Floyd and Schuster 1990).

**Oxyethira grisea* Betten. Davis Br, Bell Co; Beaver Crk and Eagle Crk, McCreary Co; Schoolhouse Hollow Spring (LBL), Trigg Co; April, June, October. Range: widely distributed in eastern U.S. and Canada.

**Oxyethira novasota* Ross. Blood River, Calloway Co; Cogur Frk, McCreary Co; April. Range: AL, AR, FL, GA, KY, LA, MS, NJ, OH, SC, and TX.

Oxyethira pallida (Banks). Scattered localities statewide; May–September (Resh 1975). This species is the most widespread *Oxyethira* in Kentucky, with records from lentic and lotic habitats.

+*Oxyethira pescadori* Harris & Keth. Piney Frk Crk (Fort Campbell), Montgomery, TN

(Christian Co, Kentucky); July–August (Etnier et al. 2006). Range: AL, FL, KY, TN, and VA. Rank: G3G4. Proposed KY rank and status: S1S2, S.

+**Oxyethira rivicola* Bickle and Morse. Sinking Crk system, Laurel Co; July–August. Range: widely distributed in eastern U.S. and Canada. Rank: G5. Proposed KY rank and status: S1S2, T. Johansen (2000) provided the only KY records for this species during his intensive survey of the Sinking Crk basin in Laurel Co.

+**Oxyethira rossi* Bickle and Morse. Big South Frk Cumberland River (BISO), McCreary Co; May, July–August. Range: KY, ME, MN, NH, TN, and WI. Rank: G3G4. Proposed KY rank and status: S1S2, T.

Oxyethira zeronia Ross. Sinking Crk system, Laurel Co; Buck Crk, Pulaski Co; unnamed trib Dog Frk, Wolfe Co (Jones 2000); June–September (Floyd and Schuster 1990).

Genus *Stactobiella*. The genus is represented by five species in North America; three species are known from KY. The larvae occupy rocks in swiftly flowing sections of small streams.

Stactobiella delira (Ross). Clemons and Coles Frk (Robinson Forest), Breathitt Co; Sinking Crk system, Laurel Co; Salt Lick Crk, Marion Co; Cumberland River and Laurel Crk, McCreary Co; Brushy Crk and Buck Crk, Pulaski Co; Horselick Crk, Rockcastle Co; Bark Camp Crk, Whitley Co; April–May (Resh 1975).

Stactobiella martynovi Bickle and Denning. Three widely scattered localities: Bad Br SNP, Letcher Co; Piney Frk Crk (Ft. Campbell Mil. Res.), Montgomery Co, TN (Christian Co., Kentucky); Clear Crk, Woodford Co; June (Houp 1999).

Stactobiella palmata (Ross). Scattered localities in Bluegrass, eastern Pennyroyal, and Eastern Coalfield: Anderson, Bell, Breathitt, Bullitt, Johnson, Laurel, Madison, McCreary, Pulaski, Spencer, Wayne, Whitley, and Woodford counties; April–September (Resh 1975).

Family Lepidostomatidae

Within North America, the family is represented by two genera, *Lepidostoma* and *Theliopsyche*, both of which have representatives in KY.

Genus *Lepidostoma*. About 75 species of *Lepidostoma* have been reported from North America (Wiggins 1996). The larvae construct distinctive, four-sided cases composed of quadrate plant or bark pieces. No keys are available for *Lepidostoma* larvae, but Weaver (1988) provided adult descriptions for all Kentucky species.

+**Lepidostoma carrolli* (Flint). Schoolhouse Hollow Spring and Turkey Spring (LBL), Trigg Co; October. Range: AR, CT, KY, MD, ME, NC, NJ, OH, PA, SC, TN, and VA. Rank: G5. Proposed KY rank and status: S1S2, S.

+**Lepidostoma etnieri* Weaver. Shillalah Crk (CUGA), Bell Co, September. Range: KY and TN (Grainger, Knox, and Roane counties) (Etnier et al. 1998). Rank: G1G2Q (Q = taxonomy questioned by NatureServe 2011, but we consider the taxon to be valid). Proposed KY rank and status: S1, E. Ranked as “Rare and Vulnerable” by Morse et al. (1997). Etnier (1997) evaluated the status of *L. etnieri* and determined that it did not warrant federal protection.

Lepidostoma griseum (Banks). Martins Frk (CUGA), Bell Co; Blowing Springs Cave, Jackson Co; Dry Frk, Metcalfe Co; Big Lick Br, Pulaski Co; unnamed trib Dog Frk, Wolfe Co (Jones 2000); September–October (Resh 1975).

+**Lepidostoma lydia* Ross. Green River, Edmonson Co; June. Range: limited to one KY locality but widely distributed in eastern U.S. and Canada (GA, KY, MA, NC, NF, NH, NJ, NS, NY, OH, PA, QC, SC, TN, VA, and VT). Rank: G5. Proposed KY rank and status: S1S2, S.

Lepidostoma pictile (Banks). Buck Crk, Pulaski Co; April (Floyd and Schuster 1990). Range: widely distributed in eastern U.S. and Canada.

+**Lepidostoma sackeni* (Banks). Upper Houchins Ferry Road (MACA), Edmonson Co. April. Range: widespread in eastern U.S. and Canada (CT, KY, MA, ME, MI, NC, ND, NF, NH, NS, NY, OH, ON, PA, QC, VT, WI, and WV). Rank: G5. Proposed KY rank and status: S1S2, S.

Lepidostoma togatum (Hagen). Scattered localities in Pennyroyal, Central Appalachians, and Southwestern Appalachians: Breathitt, Christian, Edmonson, Green, Harlan, Hart,

Laurel, Letcher, McCreary, Pulaski, Rockcastle, and Whitley counties; April–August (Resh 1975).

Genus *Theliopsyche*. The genus is represented by six species, all of which are restricted to the Appalachian Mountains and are locally distributed. Only one species, *Theliopsyche melas* Edwards is known from KY. Larvae of *Theliopsyche* construct smooth, rock cases and occupy small, spring-fed streams. No keys are available for *Theliopsyche* larvae, but an adult description of *T. melas* was provided by Edwards (1956).

Theliopsyche melas Edwards. Fayette Co. (no specific location provided by Resh [1975] – record questionable, not verified); Blue Heron Campground (BISO), McCreary Co; Big Everidge Hollow (Lily Cornett Woods), Letcher Co (Pond 2000); unnamed trib Dog Frk, Wolfe Co (Jones 2000); May–July, September (Resh 1975). Range: AL, KY, TN, VA, and WV.

Family Leptoceridae

Members of the Family Leptoceridae (long-horned caddisflies) represent some of the most widespread and abundant caddisflies in the Commonwealth. Within Kentucky, they are third behind the Hydroptilidae and Hydropsychidae in the number of species represented. Larval keys are available for all genera (see below); Ross (1944), Schmid (1980) and Moulton and Stewart (1996) provide keys and descriptions of adults.

Genus *Ceraclea*. The genus is represented by about 39 species in North America; 16 species are known from KY. The larvae construct cases of both mineral and plant fragments, and they are found in a variety of habitats. Most species are detritivores, but a few species feed on freshwater sponges (Resh 1976). Morse (1975) and Ross (1944) provided adult descriptions for most Kentucky species. Larvae of most eastern species were described by Resh (1976).

+**Ceraclea alabamae* Harris. Big South Frk Cumberland River (BISO), McCreary Co; April–May, July–August. Range: AL, KY, PA, and TN. Rank: G1G3. Proposed KY rank and status: S1S2, T. Ranked as “Rare and Vulnerable” by Morse et al. (1997).

Ceraclea ancylus (Vorhies). Widely distributed in large streams and rivers of Eastern

Coalfield, eastern Interior Plateau, with isolated records from Red River system (Logan Co) and John James Audubon State Park (Henderson Co); May–September (Resh 1975).

Ceraclea cancellata (Banks). Widely distributed in large streams and rivers of Eastern Coalfield and eastern Interior Plateau, with isolated records from Red River system (Logan Co) and John James Audubon State Park (Henderson Co); May–September (Resh 1975).

Ceraclea diluta (Hagen). Overall Crk and Wilson Crk (Bernheim Forest), Bullitt Co; Green River, Green Co; Salt Lick Crk, Marion Co; and Eagle Crk, Owen Co; May–August (Houp 1999).

+**Ceraclea enodis* Whitlock and Morse. Unnamed pond (Wandering Woods property, MACA), Edmonson Co; July. Range: CT, GA, IL, KY, NC, ON, SC, and VA. Rank: G4. Proposed KY rank and status: S1S3, S.

Ceraclea flava (Banks). Cumberland River, Bell Co; Green River, Hart Co; Kinniconick Crk, Lewis Co; Big South Frk Cumberland River (BISO), McCreary Co; South Frk Licking River, Pendleton Co; Rockcastle River, Rockcastle Co; April–August (Resh 1975).

Ceraclea maculata (Banks). Scattered localities statewide; May–September (Resh 1975).

Ceraclea neffi (Resh). Robinson Forest, Breathitt Co; Sinking Crk system, Laurel Co; South Frk Licking River, Pendleton Co; Horselick Crk, Rockcastle Co; June–August (Resh 1975).

Ceraclea nepha (Ross). Scattered localities in Interior Plateau, Southwestern Appalachians, and Jackson Purchase; Bullitt, Calloway, Clinton, Graves, Hickman, Laurel, Marshall, Pulaski, and Trigg counties (May–June; Floyd and Schuster 1990).

Ceraclea ophioderus (Ross). Barren River, Warren Co.; July (Houp 1999). Range: widely distributed in eastern U.S. (AL, AR, FL, IL, IN, KY, LA, MO, MS, NC, TX, VA, and WV).

Ceraclea protonephra Morse and Ross. Scattered localities in Central and Southwest Appalachians, Pennyroyal, and Jackson Purchase; Bell, Calloway, Clinton, Graves, Hickman, Jackson, Marshall, McCreary, Pulaski, Trigg, and Wayne counties; May–July (Floyd and Schuster 1990).

Ceraclea punctata (Banks). Scattered localities in Licking River basin and western Pennyroyal: Bath, Christian, Kenton, Pendleton, and Rowan counties; larvae (Houp and Schuster 1997).

Ceraclea resurgens (Walker). Harrods Crk, Jefferson Co; Cumberland River, McCreary Co; Buck Crk, Pulaski Co; April–June (Resh 1975).

+**Ceraclea spongillivorax* (Resh). Unnamed pond (Blue Heron campground, BISO), McCreary Co; May, July, September. Range: AL, FL, IL, IN, KS, KY, LA, MD, MS, and VA. Rank: G3G4. Proposed KY rank and status: S1S2, T.

Ceraclea tarsipunctata (Vorhies). Statewide; May–July (Resh 1975).

Ceraclea transversa (Hagen). Scattered localities statewide, except Western Allegheny Plateau and Western Coalfield; May–September (Resh 1975).

Genus *Leptocerus*. A single species occurs in North America, *Leptocerus americanus* (Banks). *Leptocerus* larvae live among aquatic plants in lentic habitats, where they construct transparent, silken cases. Adults and larvae of the only North American species, *Leptocerus americanus* (Banks), were described by Ross (1944).

Leptocerus americanus (Banks). Scattered localities in western Pennyroyal and Jackson Purchase: Adair, Calloway, Christian, Edmonson, Graves, Hart, Hickman, and Trigg counties, with one additional record from Hart Co, 100-acre pond; May–August (Etnier et al. 2006).

Genus *Mystacides*. One of only three North American species, *Mystacides sepulchralis* (Walker), occurs in Kentucky. The larval case is composed of rock or plant material, with twigs or conifer needles protruding from the case. Adults and larvae are illustrated in Ross (1944).

Mystacides sepulchralis (Walker). Statewide; May–October (Resh 1975).

Genus *Nectopsyche*. About 15 species occur north of Mexico; four species are known from KY. Adult descriptions of *Nectopsyche* were provided by Ross (1944) and Haddock (1977); eastern larvae were described by Glover and Floyd (2005).

**Nectopsyche albida* (Walker). Farm pond, Fayette Co; larvae. Range: widely distributed in northern and eastern North America.

Nectopsyche candida (Hagen). Scattered localities statewide; May–September (Resh 1975).

Nectopsyche exquisita (Walker). Statewide; May–October (Resh 1975).

Nectopsyche pavida (Hagen). Scattered localities in Eastern Coalfield and Central Pennyroyal: Bell, Breathitt, Edmonson, Green, Hart, Logan, McCreary, Owsley, Pulaski, Rockcastle, and Warren, and Wayne counties, with one record from a Bluegrass locality, Elk Lick Crk, Fayette Co; May–September (Resh 1975).

Genus *Oecetis*. Approximately 25 species are known from North America north of Mexico. The larvae are predaceous and construct a variety of cases out of rock and plant materials. Adults of Kentucky species were described by Ross (1941, 1944, 1966); Floyd (1995) provided larval descriptions of all Kentucky species except *O. ditissa* and *O. scala*.

Oecetis avara (Banks). Eastern Coalfield, eastern Interior Plateau, and isolated records from Christian Co and Hickman Co; June–August (Resh 1975).

Oecetis cinerascens (Hagen). Statewide; May–October (Resh 1975).

Oecetis ditissa Ross. Statewide; May–October (Resh 1975).

Oecetis inconspicua (Walker). Statewide; April–October (Resh 1975). One of the most widely distributed caddisfly species in KY and North America. Based on larval and adult associations made by Floyd (1995) and genetic evidence provided by Zhou et. al. (2011), *O. inconspicua* appears to be a species complex with an undetermined number of species.

Oecetis nocturna Ross. Statewide; May–October (Resh 1975).

Oecetis persimilis (Banks). Statewide; June–October (Resh 1975).

+*Oecetis scala* Ross. Big South Frk Cumberland River (BISO), McCreary Co; April–August (Houp 1999). Range: locally distributed in AL, AR, KY, MD, NC, NH, NJ, PA, QC, and SC. Rank: G4G5. Proposed KY rank and status: S1S2, T.

+**Oecetis sphyrta* Ross. Big South Frk Cumberland River (BISO), McCreary Co; July. Range: widely distributed in the southeastern U.S. (AL, FL, GA, KY, LA, MS, NC, SC, TN, TX, and VA). Rank: G5. Proposed KY rank and status: S1S2, T.

Genus *Setodes*. Nine species are known from North America; only two species have been found in KY. Holzenthal (1982) provided adult descriptions and distributional information for all North American species; Nations (1994) provided larval descriptions of both Kentucky species.

+*Setodes epicampes* Edwards. Red River, Logan Co; unnamed trib Casey Crk (Fort Campbell), Trigg Co; June (Etnier et al. 2006). Range: AL, KY, and TN. Rank: G2. Proposed KY rank and status: S2, S.

Setodes incertus (Walker). Cumberland River, Bell Co; unnamed spring (MACA), Edmonson Co; Green River, Green and Hart counties; 100-acre Pond, Hart Co; South Knob Crk (ABLI), Larue Co; Big South Frk Cumberland River (BISO), McCreary Co; June–September (Resh 1975).

Genus *Triaenodes*. The genus is represented by 22 species in North America, 12 of which are known from KY. The slender, tapered larval cases are constructed of spirally arranged plant pieces. Glover (1996) provided larval descriptions of all Kentucky species except *Triaenodes dipsius* Ross. Manuel (2010) reviewed the adults of all North American species, including the description of several new species.

Triaenodes aba Milne. Scattered localities across KY: Elliott, Graves, Henderson, Hickman, Jefferson, Johnson, Scott, and Wayne counties; May, July (Resh 1975).

+**Triaenodes cumberlandensis* Etnier and Way. Grindstone Crk, Calloway Co; June. Range: AL, AR, GA, KY, OK, and TN. Rank: G3G4. Proposed KY rank and status: S1S3, S.

+**Triaenodes dipsius* Ross. Clemons Frk (Robinson Forest), Breathitt Co; June. Range: AL, AR, KS, KY, MN, ND, OH, OK, PA, QC, TN, VA, and WI. Rank: G5. Proposed KY rank and status: S1S2, T.

Triaenodes flavescens Banks. No specific locality, Bell Co; Big Sandy River, Boyd Co; no date provided (Resh 1975). The KDOW provided numerous larval (KDOW) records of this species, but until larval vouchers can be located or adults are collected from some of these same sites, we will assume that the species is restricted to the Boyd Co locality.

Triaenodes ignitus (Walker). Statewide; May–July (Resh 1975).

Triaenodes injustus (Hagen). Statewide; May–September (Resh 1975).

Triaenodes marginatus Sibley. Scattered localities statewide: Bell, Breathitt, Calloway, Clay, Crittenden, Franklin, Grayson, Harlan, Laurel, Letcher, Martin, Metcalfe, McCreary, Pike, Trigg, and Wayne counties; April–September (Etnier et al. 2006).

Triaenodes melaca Ross. Scattered localities statewide: May–September (Resh 1975).

Triaenodes nox Ross. Saline Crk (Fort Campbell), Stewart Co, TN (Trigg Co, KY); 100-acre Pond, Hart Co; June (Etnier et al. 2006).

**Triaenodes ochraceus* (Betten and Moseley). Grindstone Crk, Calloway Co; Beaver Crk, McCreary Co; June. Range: AL, AR, CT, DE, FL, GA, KY, MS, NC, OH, SC, TN, TX, and VA.

Triaenodes perna Ross. Statewide; May–September (Houp 1999).

Triaenodes tardus Milne. Statewide; May–October (Resh 1975).

Family Limnephilidae

This family contains nearly 100 genera and 900 described species. The group has attracted the attention of scientists for many years, but much uncertainty remains about the family limits and internal organization (Vshivkova et al. 2007). The arrangement used below may change again in the near future.

Genus *Frenesia*. The genus *Frenesia* is comprised of two species, *F. difficilis* (Walker) and *F. missa* (Milne), both of which are confined to eastern North America. Larvae were described by Flint (1960); adult descriptions of *F. difficilis* and *F. missa* were provided by Betten and Moseley (1940) and Moulton and Stewart (1996), respectively. Only *F. difficilis* has been observed in Kentucky.

+**Frenesia difficilis* (Walker). Springs and spring runs at Terrapin Crk SNP, Graves Co; November–January. Range: locally distributed from NF to TN and west to KY. Rank: G5. Proposed KY rank and status: S1S2, T. Larvae of this species were first observed in KY by KSNPC and KDOW in 2000, but adults were not collected until November 2008 when Malaise traps produced over 100 individuals. The Terrapin Crk population represents a significant, western range extension for the

species as the closest, previously known populations in OH (Stillfork Swamp, Carroll County) and TN (Great Smoky Mountains National Park) are located approximately 480 to 780 km to the east (Usis and MacLean 1986; Etnier et al. 1998). Adults of this species are unusual in that they emerge during the late fall and early winter (November to January).

Genus Iroquoia. Four species of *Iroquoia* are known from North America; three of these species, *I. kaskaskia* (Ross), *I. lyrata* (Ross), and *I. punctatissima* (Walker), occur in Kentucky. Flint (1960) described the larva of *I. punctatissima* (Flint 1960), and associated material exists for the other two Kentucky species (Etnier et al. 1998; Wiggins 1996). Adult descriptions were provided by Ross (1944) and Moulton and Stewart (1996).

Iroquoia kaskaskia (Ross). Unnamed spring seep (DBNF), Bath Co; Noahs Spring (Fort Campbell), Christian Co; Sinking Crk (DBNF), Laurel Co; East Frk Clarks River, Marshall Co; Thompson Br, Simpson Co; September–October (Etnier et al. 2006). Johansen (2000) provided the first Kentucky record of this species (Sinking Crk, Laurel Co), but his record was not published.

Iroquoia lyrata (Ross). Tunnel Crk (CUGA), Bell Co; Big Sandy River, Boyd Co; June, September (Haag and Hill 1983). Range: locally distributed from QC to VA and west to WI.

Iroquoia punctatissima (Walker). Scattered localities statewide; September–October (Resh 1975).

Genus Limnephilus. The genus is represented in North America by almost 100 species. Flint (1960) described the larvae of both Kentucky species, *I. indivisus* Walker and *I. submonilifer* Walker; Ruiter (1995) provided adult descriptions. This paper provides the first records of this genus from Kentucky, but both species have been reported from neighboring states (Arkansas, Illinois, Indiana, Ohio, Tennessee, and Virginia). Larvae of these species frequent temporary ponds and wetlands.

**Limnephilus indivisus* Walker. Davis Br (CUGA), Bell Co; May, July, September. Range: widely distributed across Canada and eastern US, with records extending to UT and VA. Rank: G5. Proposed KY rank and status: S1S3, S.

**Limnephilus submonilifer* Walker. Davis Br (CUGA), Bell Co; Clear Crk, Woodford Co; April, October. Range: eastern U.S. and Canada, with records extending to TN and VA. Rank: G5. Proposed KY rank and status: S1S3, S.

Genus Platycentropus. Only one of the three Nearctic species, *P. radiatus* (Say), occurs in Kentucky. Flint (1960) described the larva; adult descriptions were provided by Ross (1944), Schmid (1980), and Moulton and Stewart (1996). We have records of *P. radiatus* from two very different habitats, a natural wetland habitat in western Kentucky (Murphys Pond, Hickman County) and a cool mountain stream in southeastern Kentucky (Letcher County). These extremes support the general habitat description provided by Wiggins (1996), who reported that *Platycentropus* larvae can occupy a wide variety of habitat types and temperature regimes.

Platycentropus radiatus (Say). Murphys Pond, Hickman Co; Colliers Crk, Letcher Co; May–June (Resh 1975).

Genus Pseudostenophylax. Only one of two eastern species, *Pseudostenophylax uniformis* (Betten), occurs in Kentucky. Larvae frequent cool spring runs or small streams with intermittent flow (Wiggins 1996). Larval descriptions were provided by Flint (1960); adult genitalia were illustrated by Ross (1944) and Moulton and Stewart (1996).

Pseudostenophylax uniformis (Betten). Unnamed spring seep (Shillalah Crk WMA) and unnamed spring at Hensley Settlement (CUGA), Bell Co; unnamed spring (MACA), Edmonson Co.; Hog Camp Crk, Elliott Co; Paint Crk, Johnson Co; Bad Br (Bad Br SNP), Poor Frk, and unnamed trib to Line Frk (Lilley Cornett Woods), Letcher Co; Hell For Certain Crk, Leslie Co; unnamed trib Salt Lick Crk, Marion Co; Little Angel Spring (Clay Hill Memorial Forest), Taylor Co; unnamed trib Dog Frk, Wolfe Co; April–June (Resh 1975).

Genus Pycnopsyche. The genus is represented in North America by about 20 species. Adults, larvae, and larval cases of Kentucky species are described in Wojtowicz (1982) and Flint (1960). Identification of larvae is difficult but can be achieved for some species if the identifications are based on mature specimens, accurate locality information, and

careful use of species descriptions and keys provided in the references listed above (Etnier et al. 1998). At the present time, larvae of *P. flavata* and *P. gentilis* can be identified with the most certainty. Adult emergence generally occurs in the fall, typically from early September through October in Kentucky streams.

Pycnopsyche antica (Walker). Scattered localities statewide except Bluegrass: Bell, Boyd, Bullitt, Edmonson, Graves, Harlan, Laurel, McCreary, Metcalfe, Pulaski, Rowan, and Trigg counties. August–October (Picazzo and DeMoss 1980).

+**Pycnopsyche circularis* (Provancher). Scattered localities in Eastern Coalfield: unnamed spring seep (DBNF), Bath Co; Martins Frk (CUGA), Bell Co; Sinking Crk system (DBNF), Laurel Co; September–October. Range: northeastern Canada (NS and QC) to TN and VA. Rank: G5. Proposed KY rank and status: S1S3, S. Johansen (2000) observed this species during his intensive survey of the Sinking Crk basin in Laurel County, but the record was not published. This species is widely distributed across eastern North America (NS to TN and west to WI), but populations appear to be localized, with only a few individuals observed at each site (Wojtowicz 1982).

+**Pycnopsyche flavata* (Banks). Limited to Central Appalachians: Shillalah Crk (CUGA), Bell Co; July, September. Range: southern Appalachian Mountains of GA, KY, NC, SC, TN, and VA. Rank: G4. Proposed KY rank and status: S1S2, T. This species inhabits higher elevation seeps and streams and is one of the earliest emerging *Pycnopsyche*, with adults appearing as early as May or June in some parts of its range (Wojtowicz 1982).

Pycnopsyche gentilis MacLachlan. Scattered localities statewide except Jackson Purchase (adults and larvae); August–October (Resh 1975).

Pycnopsyche guttifer (Walker). Scattered localities in Southwestern Appalachians and Interior Plateau: Bell, Bullitt, Edmonson, Fayette, Jefferson, Laurel, Pulaski, Simpson, and Woodford counties; September–October (Resh 1975). The Beargrass Crk population (Jefferson Co) is likely extirpated due to water quality and habitat degradation.

Pycnopsyche indiana (Ross). Scattered localities in Eastern Coalfield and Interior

Plateau: Bath, Bell, Boyd, Bullitt, Fayette, Hart, Jessamine, Menifee, Simpson, Washington, and Woodford counties; September–October (Haag and Hill 1983).

Pycnopsyche lepida (Hagen). Shillalah Crk (Shillalah Crk WMA), Bell Co; Overall Crk and Wilson Crk (Bernheim Forest), Bullitt Co; 300-Springs, Hart Co; Chalk Slough (Obion Crk WMA), Hickman Co; Sinking Crk system, Laurel Co; Covered Bridge Boy Scout Camp, Oldham Co; Buck Crk, Pulaski Co; and Thompson Br, Simpson Co; August–October (Resh 1975).

Pycnopsyche luculenta (Betten). Scattered localities in Pennyroyal and Eastern Coalfield: Davis Br, Martins Frk, and Shillalah Crk (CUGA), Bell Co; Big Sandy River, Boyd Co; Cope Frk, Breathitt Co; cinnamon fern bog (MACA), Edmonson Co; Sinking Crk system, Laurel Co; Dry Frk system, Metcalfe Co; Big Lick Br (DBNF), Pulaski Co; Thompson Br, Simpson Co; unnamed trib Dog Frk, Wolfe Co; September–October (Haag and Hill 1983).

+**Pycnopsyche rossi* Betten. Springs and spring-fed streams of Pennyroyal: unnamed spring-fed trib Lynn Camp Crk, Hart Co; Buffalo Spring and Doe Run, Meade Co; Dry Frk system, Metcalfe Co; September–October. Range: AR, IN, IL, KY, MO, OH, and TN (Wojtowicz 1982; Moulton and Stewart 1996). Rank: G3. Proposed KY rank and status: S2S3, S. This species is restricted to springs and spring-fed streams across its range.

+**Pycnopsyche subfasciata* (Say). Elk Lick Crk, Fayette Co; 300-Springs, Hart Co; September–October. Range: widely distributed, with records from Quebec south to GA and west to CO and AB (Wojtowicz 1982). Rank: G5. Proposed KY rank and status: S1S3, S.

+**Pycnopsyche virginica* (Banks). Noahs Spring, Christian Co; East Frk Clarks River (Clarks River NWR), Marshall Co; October (Etnier et al. 2006). Range: locally distributed in AL, KY, NC, SC, TN, and VA (Wojtowicz 1982; Flint et al. 2008). Rank: G3G4. Proposed KY rank and status: S1S3, T. This species is the rarest *Pycnopsyche*, with few adult collections across its range (Wojtowicz 1982). Flint et al. (2008) commented that adults in VA have been taken only in the Coastal Plain near small, spring-fed streams.

Family Molannidae

The Molannidae is a small family of the Oriental and Holarctic faunas, having only two genera and about three dozen species. Both genera occur in North America, but only one occurs in Kentucky. Larvae are instantly recognized by their distinctive shield-shaped cases, made of a central tube of fine sand grains with lateral flanges and a prolonged, anterior hood or cowl made of larger sand grains or gravel. Larvae are completely hidden by the flanges and hood when moving and feeding. In this sense, they resemble certain larvae of *Ceraclea* (Leptoceridae), but they differ in details of case construction and in their habitats.

Genus *Molanna*. Six species of *Molanna* have been reported from North America. The larvae construct distinctive, flattened cases with an anterior hood and lateral flanges. Sherberger and Wallace (1971) provided larval keys for all Kentucky species; Ross (1944) and Roy and Harper (1980) provided illustrations of males and females, respectively.

Molanna blenda Sibley. Spring-fed streams of Eastern Coalfield, southern Pennyroyal, and Jackson Purchase: Allen, Barren, Bell, Breathitt, Clay, Edmonson, Elliott, Graves, Harlan, Hart, Laurel, Letcher, McCreary, Metcalfe, Owsley, Trigg, Whitley, and Wolfe counties; May–October (Houp 1999). Zhou et al. (2011) provided evidence that *M. blenda* is a species complex composed of morphologically similar forms with substantial genetic divergence.

**Molanna tryphena* Betten. Blood River, Calloway Co; Cogur Frk (DBNF), McCreary Co.; April, June. Range: QC to AL.

**Molanna ulmerina* Navas. Big South Frk Cumberland River (BISO) and Cogur Frk (DBNF), McCreary Co; April–August. Range: southern Canada south to FL and west to AR.

Family Odontoceridae

The Odontoceridae is a small family with just over 100 species and 14 genera with a world-wide distribution. Thirteen species and six genera are known from North America; two genera and six species are found in the study area.

Genus *Psilotreta*. Parker and Wiggins (1987) provided descriptions, keys, and distributional information for all 11 North American

species. *Psilotreta* larvae are unique among North American caddisflies based on their sturdy case construction (strongest case of any North American caddisfly) and their propensity just prior to pupation to attach their cases in dense layers on the underside of rocks.

**Psilotreta frontalis* Banks. Station Creek (CUGA), Lee Co, VA (larvae). Larvae were discovered within CUGA, just south of the KY border.

Psilotreta labida Ross. Fletchers Frk (Fort Campbell), Montgomery Co, TN (Christian Co, KY); Salt Lick Crk, Marion Co; Little South Frk, McCreary Co; Casey Crk, Trigg Co; Clear Crk, Woodford Co; May–June (Parker and Wiggins 1987).

Psilotreta rufa (Hagen). Springs or spring runs of Western Allegheny Plateau and Pennyroyal: Cooper Spring, Good Spring, and Three Springs (MACA), Edmonson Co; Cooper Spring (MACA), Hart Co; Little Angel Spring (Clay Hill Memorial Forest), Taylor Co; Schoolhouse Hollow Spring (LBL), Trigg Co; unnamed trib Dog Br (DBNF), Wolfe Co; May–June (Resh 1975).

Family Philopotamidae

Larvae of Philopotamidae are restricted to lotic habitats where they construct elongate, sac-like nets on the underside of rocks. The fine-mesh nets are used to filter the water, and trapped food particles are removed from the net with the larva's highly specialized (T-shaped), membranous labrum. Lago and Harris (1987) and Armitage (1991) provided adult descriptions, keys, and distributional information for adults of all North American species.

Genus *Chimarra*. The genus *Chimarra* is represented by 20 species in North America north of Mexico. Ross (1944) provided larval descriptions and a key to all four Kentucky species.

Chimarra aterrima Hagen. Statewide; April–September (Resh 1975).

Chimarra feria Ross. Scattered localities of western Pennyroyal and Jackson Purchase: Grindstone Crk, Calloway Co; Terrapin Crk SNP, Graves Co; Thompson Br, Simpson Co; Arlt Spring and Crooked Crk (LBL), Trigg Co; May–July, October (Resh 1975).

Chimarra obscura (Walker). Statewide; May–October (Resh 1975).

Chimarra socia Hagen. Trammel Frk, Allen Co; Salt River, Anderson Co; Cumberland River, Bell Co; Big South Frk Cumberland River (BISO) and Cogur Frk (DBNF), McCreary Co; May–July (Resh 1975).

Genus *Dolophilodes*. The genus is represented by 10 species in North America. Ross (1944) described the larva of the only Kentucky species, *Dolophilodes distincta* (Walker).

Dolophilodes distincta (Walker). Statewide, except northern Bluegrass and Jackson Purchase; February–October (Resh 1975).

Genus *Fumonta*. Blahnik (2005) elevated the monotypic subgenus *Fumonta* of *Dolophilodes* to generic status. Adult illustrations of *F. major* (Banks) were provided by Ross (1956), and Weaver et al. (1981) described the larva.

+**Fumonta major* (Banks). Big Lick Br (DBNF), Pulaski Co; June. Range: Appalachian Mountains of AL, GA, KY, NC, SC, TN, and VA. Rank: G4G5. Proposed KY rank and status: S1S2, T.

Genus *Wormaldia*. The genus is represented in Kentucky by only 3 of the 14 North American species. Ross (1944) described the larvae of *Wormaldia moesta* (Banks) and *W. shawnee* Ross. The larva of *W. thyria* is unknown.

Wormaldia moesta (Banks). Cool, spring-fed streams of Eastern Coalfield and Interior Plateau; March–October (Resh 1975).

Wormaldia shawnee Ross. Scattered localities in Southwestern Appalachians and Interior Plateau: Allen, Bullitt, Christian, Grant, Laurel, Pulaski, Taylor, and Woodford counties; April–June, August (Resh 1975).

+**Wormaldia thyria* Denning. Pine Mountain Settlement School and Watts Crk (Blanton Forest SNP), Harlan Co; Yahoo Crk and Big South Frk Cumberland River (BISO), McCreary Co; June. Range: Appalachian Mountains of KY, NC, SC, TN, and VA. Rank: G3. Proposed KY rank and status: S1S2, T. All KY populations occur in small, undisturbed streams with good water quality.

Family Phryganeidae

The Phryganeidae, or giant caddisflies, include some of the largest species (over 40 mm long) in North America. The larvae are more active than other case-making caddisflies and are less dependent on their case, which they may abandon when threatened (Wiggins 1996). The adults are strong fliers

and appear to be attracted to sugar solutions, as evidenced by their capture in fermenting molasses traps in Arkansas (Bowles et al. 1990). Wiggins (1998) provided detailed descriptions, keys, and distributional information for adults and some larvae of North American genera and species. The family is represented in Kentucky by five genera and eight species.

Genus *Agrypnia*. This Holarctic genus contains 17 species, with 10 species in North America and one species in KY. Larvae prefer habitats with still or slowly moving water.

Agrypnia vestita (Walker). Scattered localities statewide: Anderson, Bath, Bell, Edmonson, Fayette, Graves, Hart, Meade, Metcalfe, Pulaski, Trigg, Wolfe, and Woodford counties; April–October (Resh 1975).

Genus *Banksiola*. This genus is comprised of five species, all of which are restricted to North America. Only one species is known from KY.

Banksiola dossuaria (Say). Central Appalachians: Martins Frk and Shillalah Crk (CUGA), Bell Co; Bad Br SNP, Letcher Co; Shelby Gap, Pike Co; June–July (Resh 1975). Range: widely distributed in eastern North America (LB to SC to WI).

Genus *Oligostomis*. This is a Holarctic genus of five species, two of which are restricted to North America. Only one species is known from the study area. The omnivorous larvae inhabit cool streams in areas of slow current and accumulated leaves.

+**Oligostomis ocelligera* (Walker). Dry Br (MACA), Edmonson Co; April. Range: northeastern U.S. and Canada, with isolated records from IN and TN. Rank: G5. Proposed KY rank and status: S1S3, S.

Genus *Phryganea*. This is a Holarctic genus of eight species, two of which are found in North America and KY. The omnivorous larvae prefer lake and marsh habitats.

+**Phryganea cinerea* (Walker). Good Spring (MACA), Edmonson Co; April, June, August. Range: northern U.S. and Canada (Wiggins 1998), with isolated records in Clark County, IN (Waltz and McCafferty 1983) and KY. Rank: G5. Proposed KY rank and status: S1S3, S. Its collection in Kentucky represents a southern range extension.

Phryganea sayi Milne. Scattered localities statewide: Breathitt, Bullitt, Edmonson, Fayette, Graves, Larue, Laurel, Marion, Marshall,

McCreary, Meade, Oldham, Pendleton, Pulaski, Spencer, and Whitley counties; May, July–September (Resh 1975).

Genus *Ptilostomis*. The genus is restricted to North America, with a total of four species. Three of these species are known from KY. Species of *Ptilostomis* are considered “ecological generalists,” particularly *P. ocellifera* (Wiggins 1998). Larvae are found in a wide range of habitats from spring streams to temporary pools. Adults of all four species are similar in appearance and are less ornamentally marked than other phryganeid genera. Larvae cannot be separated at this time.

Ptilostomis ocellifera (Walker). Grindstone Crk, Calloway Co; Wet Prong (MACA), Edmonson Co; Sinking Crk system, Laurel Co; no specific locality, Oldham Co; unnamed trib Dog Frk, Wolfe Co; May–July (Resh 1975).

Ptilostomis postica (Walker). Scattered localities statewide: Bath, Bullitt, Edmonson, Graves, Hart, Hickman, Larue, Letcher, Marshall, McCreary, and Pulaski counties; April–June, September–October (Floyd and Schuster 1990).

Ptilostomis semifasciata (Say). Cumberland River, Bell Co; Coles Frk (Robinson Forest), Breathitt Co; White Oak Crk, Laurel Co; June, August (Resh 1975).

Family Polycentropodidae

Larvae of Polycentropodidae construct a variety of fixed, silken retreats, many of which have outlying silken strands that allow the larva to detect the vibrations of potential prey. Larval descriptions are lacking for most North American species, but Ross (1944) provided larval descriptions and keys for some Kentucky species. Armitage and Hamilton (1990) provided descriptions, keys, and distributional information for North American adults.

Genus *Cernotina*. Only seven species are known from North America, north of Mexico; one species occurs in Kentucky.

Cernotina spicata Ross. Scattered localities in Eastern Coalfield, western Pennyroyal, and Jackson Purchase: Bell, Christian, Graves, Laurel, and Trigg counties; May–August (Etnier et al. 2006).

Genus *Cyrnellus*. Only one species, *Cyrnellus fraternus* (Banks), is known from

North America and is widely distributed in the eastern US.

Cyrnellus fraternus (Banks). Statewide; June–September (Resh 1975).

Genus *Neureclipsis*. Five species are known from North America. Larvae of *Neureclipsis* construct distinctive, trumpet shaped nets (up to 10 cm in length) that are attached to fixed objects in areas with slow current.

Neureclipsis crepuscularis (Walker). Scattered localities in Eastern Coalfield, Pennyroyal, and Jackson Purchase; May–August (Resh 1975).

Neureclipsis parvula (Banks). Cumberland River, Bell Co; Cumberland River, Lyon Co; June–August (Resh 1975).

+*Neureclipsis piersoni* Frazer and Harris. Noahs Spring (FCMR), Christian Co; Big South Frk Cumberland River (BISO), McCreary Co; unnamed trib Casey Crk (FCMR), Trigg Co; no date (Etnier et al. 2006). Range: AL, GA, KY, and TN. Rank: G1G3. Proposed KY rank and status: S1S3, S.

Genus *Nyctiophylax*. About 10 species of *Nyctiophylax* have been reported from North America; 6 species are reported from Kentucky, including 3 species for the first time.

Nyctiophylax affinis (Banks). Statewide; May–September (Resh 1975).

+*Nyctiophylax banksi* Morse. White Oak Crk, Laurel Co; June–August. Range: AL, CT, KY, ME, MA, MN, MS, ON, PA, QC, SC, TN. Rank: G4G5. Proposed KY rank and status: S1S3, T. Johansen (2000) provided the first and only Kentucky record of this species, but the record was not published.

Nyctiophylax celta Denning. Big South Frk Cumberland River (BISO), McCreary Co; Cumberland River, Whitley Co; unnamed trib Dog Frk, Wolfe Co; August (Resh 1975).

**Nyctiophylax moestus* Banks. Shillalah Crk and Sugar Run (CUGA), Bell Co; Clemons Frk, Breathitt Co; Grindstone Crk, Calloway Co; Good Spring and Wet Prong (MACA), Edmonson Co; Sinking Crk System, Laurel Co; Bad Br (Bad Br SNP) and Big Everidge Hollow (Lilley Cornett Woods), Letcher Co; Salt Lick Crk, Marion Co; Eagle Crk and Yahoo Crk (BISO), McCreary Co; Dry Frk, Metcalfe Co; May–August. Range: widely distributed in northern and eastern North America.

Nyctiophylax serratus Lago and Harris. Murphys Pond and East Frk Clarks River

(Clarks River NWR), Marshall Co; Saline Crk, Stewart Co, TN (Trigg Co); May–June (Etnier et al. 2006). Range: AL, AR, FL, KY, MS, MO, TN, TX, and VA.

Nyctiophylax uncus Ross. No specific locality reported by Morse (1972), who reported the species from eastern Kentucky (Resh 1975). Range: widely distributed in the eastern U.S. and Canada from ON and QC south to KY and TN.

Genus *Polycentropus*. The genus *Polycentropus* is represented by about 50 species in North America (Wiggins 1996) and 16 species in Kentucky. Ross (1944) provided larval descriptions for six species, but larval-adult associations are lacking for most species.

+*Polycentropus barri* Ross and Yamamoto. Robinson Forest, Breathitt Co; Blowing Springs Cave and John Rogers Cave (DBNF), Jackson Co; May–July (Resh 1975). Range: AL, KY, PA, and TN. Rank: G2G4. Proposed KY rank and status: S1S2, T.

Polycentropus blicklei Ross and Yamamoto. South Knob Crk (ABLI), Larue Co; Salt Lick Crk, Marion Co; Arlt Spring (LBL) and Lake Barkley, Trigg Co; April–June (Resh 1975).

+**Polycentropus carolinensis* Banks. Dark Ridge Br and Shillalah Crk (CUGA), Bell Co; unnamed springs (MACA), Edmonson Co; unnamed trib Dog Br, Wolfe Co; April, July. Range: eastern North America from QC to TN and VA. Rank: G5. Proposed KY rank and status: S2S3, S.

Polycentropus centralis Banks. Scattered localities of Southwestern Appalachians and Interior Plateau: Bullitt, Calloway, Christian, Edmonson, Fayette, Franklin, Larue, Laurel, Madison, Marion, Marshall, Pulaski, Simpson, Taylor, and Trigg counties; April–October (Floyd and Schuster 1990).

Polycentropus chelatus Ross and Yamamoto. Harts Run and Overall Crk (Bernheim Forest), Bullitt Co; Little Angel Spring (Clay Hill Memorial Forest), Taylor Co; unnamed trib Casey Crk, Trigg Co; May (Etnier et al. 2006).

Polycentropus cinereus Hagen. Statewide; May–October (Resh 1975).

+**Polycentropus colei* Ross. Shillalah Crk (Shillalah Crk WMA and CUGA), Bell Co; June. Range: KY, NC, PA, QC, TN, and WV. Rank: G3G4. Proposed KY rank and status: S1S2, T.

Polycentropus confusus Hagen. Interior Plateau and Eastern Coalfield: Bell, Bullitt, Edmonson, Elliott, Fayette, Harlan, Laurel, Letcher, Marion, McCreary, Metcalfe, Pulaski, Rockcastle, and Simpson counties; May–October (Resh 1975).

Polycentropus crassicornis Walker. Camp Breckinridge, Breckinridge Co; East Frk Clarks River (Clarks River NWR), Marshall Co; Arlt Spring (LBL), Trigg Co; May (Resh 1975).

Polycentropus elarus Ross. Interior Plateau and Eastern Coalfield: Barren, Bell, Breathitt, Bullitt, Edmonson, Elliott, Fayette, Franklin, Laurel, Letcher, Marion, Meade, Metcalfe, Pulaski, Taylor, and Wayne counties; April–October (Resh 1975).

Polycentropus maculatus Banks. High quality, cool streams of Central Appalachians and Western Allegheny Plateau: Bell, Breathitt, Harlan, Letcher, and Wolfe counties; May–August (Resh 1975).

+**Polycentropus nascotius* Ross. Eastern Coalfield: Davis Br and Shillalah Crk (CUGA), Bell Co; Watts Crk (Blanton Forest SNP), Harlan Co; Blue Heron Campground (BISO), McCreary Co; May–July, September. Range: locally distributed in eastern North America (MN to NS south to OK and AL). Rank: G5. Proposed KY rank and status: S2S3, S.

+*Polycentropus neiswanderi* Ross. Salt Lick Crk, Marion Co; April (Houp 1999). Range: KY and OH. Rank: G1G3. Proposed KY rank and status: S1S2; KY Status: T.

+**Polycentropus pentus* Ross. Shillalah Crk (CUGA), Bell Co; unnamed trib Camp Pleasant Br, Franklin Co; unnamed trib Dog Br, Wolfe Co; May–June, August. Range: eastern North America (MB to NS, south to AL), with one outlying record from WY. Rank: G5. Proposed KY rank and status: S1S3, S. This species was first observed in Kentucky (Wolfe Co) by Jones (2000), but his record was not published. The species appears to be limited to small, spring seeps and spring-fed first order streams.

Polycentropus remotus Banks. Salt River, Spencer Co; no date provided (Resh 1975). Range: widely distributed across Canada and eastern US; KY represents a southern extension of its range. The only Kentucky record of *P. remotus* was provided by Resh (1975), who based the record on an immature ("imm"). Until adults of this species are located, the

presence of this species within Kentucky should be considered tentative.

+**Polycentropus rickeri* Yamamoto. Shillalah Crk (CUGA), Bell Co; June, September. Range: Appalachian Mountains in AL, KY, PA, TN, and VA. Rank: G3G4. Proposed KY rank and status: S1S2, T.

Family Psychomyiidae

Adult descriptions, keys, and illustrations for the two Kentucky species, *Lype diversa* (Banks) and *Psychomyia flava* (Hagen), were provided by Ross (1944) and Armitage and Hamilton (1990). Larvae were described and illustrated by Flint (1964) and Wiggins (1996).

Genus *Lype*. Within North America, the genus is represented by a single species, *Lype diversa* (Banks).

Lype diversa (Banks). Scattered localities statewide except northern Bluegrass; April–October (Resh 1975).

Genus *Psychomyia*. Within North America, the genus is represented by three species, one of which, *Psychomyia flava* (Hagen), occurs in Kentucky.

Psychomyia flava (Hagen). Scattered localities statewide except Jackson Purchase; March–October (Resh 1975).

Family Ptilocolepidae

Based on taxonomic work by Malicky (2001), Morse (2011) placed the hydroptilid subfamily Ptilocolepinae in its own family. We have followed that arrangement here. Ptilocolepidae consists of two genera and 16 species, with only the genus *Palaeagapetus* found in North America.

Genus *Palaeagapetus*. This genus is represented by three North American species; the single eastern species, *P. celsus*, is reported from KY for the first time. Larvae are depressed dorsoventrally and construct unique, flattened cases constructed of small pieces of liverwort.

+**Palaeagapetus celsus* (Ross). Headwaters of Martins Frk (CUGA), Bell Co (larvae). Range: localized populations in NC, QC, TN, and VA. Rank: G5. Proposed KY rank and status: S1S2, T.

Family Rhyacophilidae

The family includes 2 genera and more than 120 species in North America; only 1 genus,

Rhyacophila, occurs in Kentucky and is represented by 15 species.

Genus *Rhyacophila*. *Rhyacophila* larvae are restricted to cool, lotic habitats where they are free-living, active predators (Wiggins 1996). Most larvae are pollution-intolerant and serve as useful biological indicators of pollution (Lenat 1993). Despite the larva's free-living lifestyle, pupation takes place within a tough, silken cocoon and loosely packed rock dome constructed by the last larval instar. Prather and Morse (2001) provided keys and descriptions for all Kentucky larvae and adults except *R. otica* Etnier and Way.

+*Rhyacophila appalachia* Morse and Ross. Robinson Forest, Breathitt Co; June (Resh 1975). Range: Appalachian Mountains in KY, NC, SC, TN, and VA. Rank: G3. Proposed KY rank and status: S1S2, E. Recent attempts to locate the species at Robinson Forest were unsuccessful.

Rhyacophila carolina Banks. Widespread in Eastern Coalfield and eastern half of Interior Plateau; April–October (Resh 1975).

Rhyacophila carpenteri Milne. Springs or spring-fed streams of Eastern Coalfield and eastern Pennyroyal: Tunnel Crk and Shillalah Crk (CUGA), Bell Co; unnamed trib Rough Br and Watts Crk (Blanton Forest SNP), Harlan Co; Bad Br SNP, Letcher Co; Dog Foot Springs, Madison Co; unnamed trib Salt Lick Crk, Marion Co; Morgans Crk, Meade Co; Buck Crk, Pulaski Co; Mill Springs Recreation Area, Wayne Co; unnamed trib Dog Frk, Wolfe Co; April–August (Resh 1975).

Rhyacophila fenestra Ross. Scattered localities in Bluegrass and Pennyroyal: Bullitt, Edmonson, Fayette, Franklin, Madison, Shelby, Simpson, Taylor, Trigg, and Trimble counties; April–May (Resh 1975).

**Rhyacophila fuscula* (Walker). Central and Southwestern Appalachians: Bell, Harlan, Letcher, Pike, and Wayne counties (adults and larvae); April–June. Range: widely distributed in eastern North America. Larvae of this species were first collected in Kentucky by KDWY personnel, but these records were not published.

Rhyacophila glaberrima Ulmer. Widely distributed statewide, except Jackson Purchase; April–May, October (Resh 1975). Zhou et al. (2011) presented genetic evidence that *R. glaberrima* is a species complex.

+**Rhyacophila invaria* (Walker). Upper Houchins Ferry Road (MACA), Edmonson Co; unnamed trib Big South Frk Cumberland River (BISO), McCreary Co (adults and larvae); April–May. Range: NF to WI and south to NC. The species' collection in Kentucky represents a southwestern range extension. Rank: G5. Proposed KY rank and status: S1S3, S.

Rhyacophila ledra Ross. Davis Br (CUGA), Bell Co; Harts Run, Bullitt Co; unnamed springs (MACA), Edmonson Co; Raven Run, Fayette Co; Terrapin Crk SNP, Graves Co; unnamed trib East Frk Clear Crk, Jessamine Co; Arlt Spring and Hematite Lake (LBL), Trigg Co; Little South Frk, Wayne Co; unnamed trib Dog Frk, Wolfe Co; May (Resh 1975).

Rhyacophila lobifera Betten. Statewide, except Western Coalfield and Jackson Purchase (adults and larvae); April–May (Resh 1975).

Rhyacophila minora Banks. Eastern Coalfield: Bell, Breathitt, Clay, Harlan, Jackson, Lee, McCreary, Menifee, Pike, and Wolfe counties; April–May (Resh 1975).

**Rhyacophila nigrita* Banks. Undisturbed, high quality streams in Central Appalachians: Shillalah Crk (CUGA), Bell Co; Martins Frk (CUGA) and Watts Crk, Harlan Co; Bad Br (Bad Br SNP), Letcher Co; June. Larvae of this species were first collected in Kentucky by KDOW personnel, but these records were not published. Zhou et al. (2011) presented evidence that *R. nigrita* represents a species complex.

+*Rhyacophila otica* Etnier and Way. This species has been documented from two Kentucky localities: Robinson Forest, Breathitt Co; unnamed seep habitats (Pine Mountain Settlement School), Harlan Co; June (Resh 1975). Range: KY, PA, TN, and VA. Rank: G3G4Q. Proposed KY rank and status: S1S2, T.

Rhyacophila parantra Ross. Scattered localities in Bluegrass, Pennyroyal, and Eastern Coalfield: Bell, Edmonson, Fayette, Franklin, Garrard, Harlan, Hart, Madison, Marion, McCreary, Meade, Taylor, Wayne, and Wolfe counties; March–August (Resh 1975).

Rhyacophila torva Hagen. Scattered localities in the eastern half of state; April–October (Resh 1975).

Rhyacophila vibox Milne. Scattered localities in Eastern Coalfield: Bell, Elliott, Laurel,

Letcher, and Morgan counties; May (Houp 1999).

Family Sericostomatidae

The family is represented by 3 North American genera and 12 species. Only one genus, *Agarodes*, has been observed in Kentucky.

Genus *Agarodes*. In the southeastern US, *Agarodes* larvae occur in springs or small spring-fed streams with sand and gravel substrates. Keth and Harris (2008) provided descriptions, illustrations, and keys to adult males and associated females and larvae of North American species.

+**Agarodes stannardi* (Ross). Four localities in western Pennyroyal: Grindstone Crk, Calloway Co.; Arlt Spring, Schoolhouse Spring, and Turkey Spring (LBL), Trigg Co (larvae, pupae, and adults); May–June. Range: AL, KY, MS, and TN (Harris et al. 1991; Keth and Harris 2008). Rank: C2G3. Proposed KY rank and status: S1S3, S. We collected one female of this species in southern Calloway County in June 2008, but attempts to collect additional individuals were unsuccessful. In June 2009, we located a second population in a spring run on LBL in Trigg County. Additional LBL populations were discovered at Schoolhouse Spring and Turkey Spring in 2010. Our collections extend the species' range approximately 160 km to the north.

Family Thremmatidae

The Family Thremmatidae is represented by 3 North American genera; only 1 genus, *Neophylax*, occurs in Kentucky and is represented by 10 species.

Genus *Neophylax*. This genus was assigned formerly to the families Limnephilidae (Ross 1944) and Uenoidae (Vineyard and Wiggins 1987; Vineyard et al. 2005), but we follow the more recent placement of the genus in Thremmatidae (Vshivkova et al. 2007). *Neophylax* larvae can be abundant in Kentucky streams during the early spring and later undergo a two- to six-month aestivation just prior to pupation (Vineyard et al. 2005). Adults typically emerge in the fall (September to October). Vineyard et al. (2005) provided keys, illustrations, and descriptions for all Kentucky larvae and adults.

+*Neophylax acutus* Vineyard and Wiggins. Red River system, Logan Co; Buck Crk system, Pulaski Co; October (Floyd and Schuster 1990). Range: AL, KY, TN, and VA. Rank: G2G3. Proposed KY rank and status: S1S3, S.

**Neophylax aniqua* Ross. Central and Southwestern Appalachians: Tunnel Crk (CUGA) and Shillalah Crk (Shillalah Crk WMA), Bell Co; unnamed trib Little Millseat Br (Robinson Forest), Breathitt Co; unnamed trib Big South Frk Cumberland River and Yahoo Crk below falls (BISO), McCreary Co (larvae). Etnier et al. (1998) reported a flight period of September–October in TN. Range: southern Canada and the northern US, with extensions down the Appalachian Mountains to KY, NC, TN, and VA.

Neophylax ayanus Ross. Scattered localities in Interior Plateau: Bear Crk, Anderson Co; Elk Lick Crk and South Elkhorn Crk, Fayette Co; unnamed trib Camp Pleasant Br, Franklin Co; Nolin River, Hardin Co; unnamed trib Lynn Camp Crk, Hart Co; Hickman Crk, Jessamine Co; Beargrass Crk system, Jefferson Co; Harrods Crk, Oldham Co; Clear Crk, Woodford Co; September–October (Resh 1975). This species was described originally from collections made by Dr. Herbert H. Ross in the 1930s at Beargrass Crk, Jefferson County (Ross 1938). Resh (1975) reported that the species was absent from Beargrass Crk during collections at the same localities in 1972; we could not find the species during collections in 2009.

Neophylax concinnus MacLachlan. Widespread in eastern two-thirds of state, absent from Western Coalfield and Jackson Purchase; October (Resh 1975).

Neophylax consimilis Betten. Limited to three localities in Eastern Coalfield: Yellow Crk (CUGA), Bell Co; Yahoo Crk below falls (BISO), McCreary Co; Fishtrap Lake, Pike Co; May, July (Resh 1975). Range: narrowly distributed along the Appalachian Mountains from NS to northern GA.

+**Neophylax etnieri* Vineyard and Wiggins. Central and Southwestern Appalachians: Davis Br and Tunnel Crk (CUGA), Bell Co; Yahoo Crk below falls (BISO), McCreary Co; May, July–September. Range: Appalachian Mountains of KY, TN, and VA. Rank: G3. Proposed KY rank and status: S1S2, T.

Neophylax fuscus Banks. Boone Crk, Clark Co; unnamed spring, 3 springs, and Green River (MACA), Edmonson Co; Fleming Co. (no specific locality); Green River, Green Co; Buck Crk, Pulaski Co (adults and larvae); October (Phillippi and Schuster 1987).

+**Neophylax lewisae* Etnier. Four sites in Pennyroyal: Bays Fork, Allen Co; Dry Frk system and Fallen Timber Creek, Metcalfe Co; Thompson Br, Simpson Co (adults and larvae); October. Range: springs or spring-fed habitats in KY and TN. Proposed rank: G2G3. Proposed KY rank and status: S1S3. S.

Neophylax mitchelli Denning. Rivals, Spencer Co (larvae) (Resh 1975). Range: southern Canada with a narrow band extending down the Appalachian Mountains to TN and VA. G3G4. We consider this record tentative because voucher specimens are not available. The Spencer Co record was based on immatures (Resh 1975), and all records from adjacent states (TN and VA) are restricted to the Appalachian Mountains.

**Neophylax wigginsi* Sykora and Weaver. Shillalah Crk (CUGA), Bell Co; Wandering Woods site (MACA), Edmonson Co; North Frk Knob Crk (ABLI), Laure Co; Yahoo Crk below falls (BISO), McCreary Co; Big Lick Br, Pulaski Co; unnamed trib Dog Frk, Wolfe Co; September–October. Range: KY, OH, PA, TN, and VA.

DISCUSSION

The 69 new distributional records represent a 30% increase in the number of Trichoptera known from Kentucky. As expected, collections from least disturbed sites or areas with unique natural or biological features (e.g., OSRWs, SNPs, natural wetlands, sinkhole ponds, and springs) supported the most diverse caddisfly faunas and also produced the greatest number of new Kentucky records. No new species were discovered during our survey efforts, but several significant range extensions were documented (e.g., *Agarodes stannardi*, *Frenesia difficilis*, and *Metricchia* sp.). We expect additional species to be found as sampling efforts continue across the Commonwealth. Unique habitats such as springs, seeps, bogs, and natural wetlands have the most potential to produce new records, and less traditional sampling techniques (e.g., Malaise traps) should be em-

Table 1. Imperiled and vulnerable Trichoptera of Kentucky.

Species	Global	KY	KY	KY County Distribution
	Rank ¹	Rank ²	Status ³	
KSNPC (2010)				
<i>Manophylax butleri</i> Schuster	G2	S2	S	Bell, Carter, McCreary, Wolfe
<i>Cheumatopsyche helma</i> Ross	G3	SH	H	Bell
Proposed				
<i>Brachycentrus nigrosoma</i> (Banks)	G5	S2S3	S	Bell, Laurel, Pulaski
<i>Micrasema scotti</i> Ross	G3G4	S1S2	T	Metcalfe
<i>Agapetus kirchneri</i> Parker, Etnier & Baxter	G2*	S1	T	Bell
<i>Protoptila alexanderi</i> Ross	G5	S1S2	T	Union
<i>Goera fuscula</i> Banks	G5	S1S2	S	Edmonson
<i>Cheumatopsyche gyra</i> Ross	G4G5	S1S2	T	Bell
<i>Hydropsyche etnieri</i> Schuster & Talak	G2	S1S2	T	Whitley
<i>Hydropsyche mississippiensis</i> Flint	G5	S2S3	S	Calloway
<i>Hydroptila ampoda</i> Ross	G5	S1S3	S	Letcher, Wolfe
<i>Hydroptila ceweetensis</i> Huryn	G1G2	S1S2	T	Breathitt, Edmonson, Laurel
<i>Hydroptila decia</i> Etnier & Way	G2	S1S2	T	Laurel
<i>Hydroptila fiskei</i> Bickle	G4G5	S1S3	T	Laurel
<i>Hydroptila howelli</i> Houp, Houp & Harris	G2G3	S1S2	T	Larue/Marion, Laurel, Menifee
<i>Hydroptila kuehnei</i> Houp, Houp & Harris	G1G2	S1S2	T	Edmonson, Larue/Marion, Meade, Metcalfe
<i>Hydroptila lennoxi</i> Bickle	G2G4	S1S3	T	Breathitt, Laurel, Wolfe
<i>Hydroptila oneili</i> Harris	G2G3	S1S2	S	Christian, Trigg
<i>Hydroptila paramoena</i> Harris	G2G3	S1S2	S	Bell, McCreary
<i>Hydroptila paraxella</i> Harris & Armitage	G3*	S1S2	S	Madison, Simpson
<i>Hydroptila sandersoni</i> Mathis & Bowles	G3G4	S1S2	S	Pulaski
<i>Hydroptila scolops</i> Ross	G4G5	S1S3	T	Bell
<i>Neotrichia riegeli</i> Ross	G3	S1S3	S	Johnson
<i>Ochrotrichia wojcickyi</i> Bickle	G4G5	S1S3	S	Fayette
<i>Orthotrichia curta</i> (Kingsolver & Ross)	G4G5	S1S2	T	Laurel
<i>Oxyethira pescadori</i> Harris & Keth	G3G4	S1S2	S	Christian
<i>Oxyethira rivicola</i> Bickle & Morse	G5	S1S2	T	Laurel
<i>Oxyethira rossi</i> Bickle & Morse	G3G4	S1S2	T	McCreary
<i>Lepidostoma carrolli</i> (Flint)	G5	S1S2	S	Trigg
<i>Lepidostoma etnieri</i> Weaver	G1G2Q	S1	E	Bell
<i>Lepidostoma lydia</i> Ross	G5	S1S2	S	Edmonson
<i>Lepidostoma sackeni</i> (Banks)	G5	S1S2	S	Edmonson
<i>Ceraclea alabamae</i> Harris	G1G3	S1S2	T	McCreary
<i>Ceraclea enodis</i> Whitlock & Morse	G4G5	S1S3	S	Edmonson
<i>Ceraclea spongillorox</i> (Resh)	G3G4	S1S2	T	McCreary
<i>Oecetis scala</i> Ross	G4G5	S1S2	T	McCreary
<i>Oecetis sphyrta</i> Ross	G5	S1S2	T	McCreary
<i>Setodes epicampes</i> Edwards	G2	S2	S	Logan, Trigg
<i>Triadenodes cumberlandensis</i> Etnier & Way	G3G4	S1S3	S	Calloway
<i>Triadenodes dipsius</i> Ross	G5	S1S2	T	Breathitt
<i>Frenesia difficilis</i> (Walker)	G5	S1S2	T	Graves
<i>Limnephilus indivisus</i> Walker	G5	S1S3	S	Bell
<i>Limnephilus submontifer</i> Walker	G5	S2S3	S	Bell, Woodford
<i>Pycnopsyche circularis</i> (Provancher).	G5	S3	S	Bath, Bell, Laurel
<i>Pycnopsyche flavata</i> (Banks)	G4	S1S2	T	Bell
<i>Pycnopsyche rossi</i> Betten	G3	S2S3	S	Hart, Meade, Metcalfe
<i>Pycnopsyche subfasciata</i> (Say)	G5	S1S3	S	Fayette, Hart
<i>Pycnopsyche virginica</i> (Banks)	G3G4	S1S2	T	Christian, Marshall
<i>Fumontia major</i> (Banks)	G4G5	S1S2	T	Pulaski
<i>Wormaldia thyria</i> Denning	G3	S1S2	T	Harlan
<i>Oligostomis ocelligera</i> (Walker)	G5	S1S3	S	Edmonson
<i>Phryganea cinerea</i> (Walker)	G5	S1S3	S	Edmonson
<i>Neureclipsis piersoni</i> Frazer & Harris	G1G3	S1S3	S	Christian, Trigg
<i>Nyctiophylax banksi</i> Morse	G4G5	S1S2	T	Laurel
<i>Polycentropus barri</i> Ross & Yamamoto	G2G4	S1S2	T	Breathitt, Jackson
<i>Polycentropus carolinensis</i> Banks	G5	S2S3	S	Bell, Edmonson

Table 1. Continued.

Species	Global	KY	KY	KY County Distribution
	Rank ¹	Rank ²	Status ³	
<i>Polycentropus colei</i> Ross	G3G4	S1S2	T	Bell
<i>Polycentropus nasctotius</i> Ross	G5	S2S3	S	Bell, Harlan, McCreary
<i>Polycentropus neiswanderi</i> Ross	G1G3	S1S2	T	Christian, Marion
<i>Polycentropus pentus</i> Ross	G5	S3	S	Bell, Franklin, Wolfe
<i>Polycentropus rickeri</i> Yamamoto	G3G4	S1S2	T	Bell
<i>Palaeagapetus celsus</i> (Ross)	G5	S1S2	T	Bell
<i>Rhyacophila appalachia</i> Morse & Ross	G3	S1S2	E	Breathitt
<i>Rhyacophila invaria</i> (Walker)	G5	S1S3	S	Edmonson, McCreary
<i>Rhyacophila otica</i> Etnier & Way	G3G4Q	S1S2	T	Breathitt, Harlan
<i>Agarodes stannardi</i> (Ross)	G2G3	S1S3	S	Calloway, Trigg
<i>Neophylax acutus</i> Vineyard & Wiggins	G2G3	S1S3	S	Logan, Pulaski
<i>Neophylax etnieri</i> Vineyard & Wiggins	G3	S1S2	T	Bell, McCreary
<i>Neophylax lewisae</i> Etnier	G2G3*	S1S3	S	Metcalfe, Simpson

¹ Global rank (NatureServe 2012): G1 (critically imperiled) G2 (imperiled) G3 (vulnerable) G4 (apparently secure) G5 (secure) and Q (taxonomy questioned); proposed ranks marked by an asterisk (*).

² KY rank: S1 (critically imperiled) S2 (imperiled) S3 (vulnerable) S4 (apparently secure) and S5 (secure).

³ KY status: E (endangered) T (threatened) S (special concern) and H (historic).

ployed to locate species that do not respond to ultraviolet light. The Licking River basin, direct tributaries of the Ohio River (e.g., Kinniconick Creek in Lewis County), and low gradient habitats in western Kentucky should be emphasized in future sampling efforts because these areas have not been sampled as extensively as other regions of the Commonwealth.

Compared to nearby states, Kentucky's fauna of 293 species is transitional, exceeding that of Illinois (183) Indiana (190), and Ohio (270) but falling short of species totals for Alabama (342), North Carolina (348), Tennessee (352), and Virginia (361). Among Kentucky's ecoregions, the Interior Plateau has the greatest number of caddisfly records (215 species), but the region also covers about half of the Commonwealth's total surface area. The Eastern Coalfield region covers about a third of Kentucky, but it exhibits even greater diversity (229 species), including 70 unique records. The lower recorded diversity of the Western Coalfield and Jackson Purchase regions is not surprising as these areas are much smaller than other regions and the western half of Kentucky has not been sampled as completely or with the same frequency as the eastern half of the Commonwealth. We expect totals for western Kentucky regions to increase with additional survey efforts.

We identify 69 species as imperiled or vulnerable in Kentucky (Table 1). Our list

includes two species, *Manophylax butleri* and *Cheumatopsyche helma*, already designated by KSNPC (2010) as Special Concern (*M. butleri*) and Historical (*C. helma*), but the list also contains Kentucky's two endemic species, *Hydroptila howelli* and *H. kuehnei*, and four other species that are known from only two states – *Hydroptila paraxella* (KY and OH), *Lepidostoma etnieri* (KY and TN), *Polycentropus neiswanderi* (KY and OH), and *Neophylax lewisae* (KY and TN). We are proposing state endangered status for two species, *L. etnieri*, which is known from only one KY locality and three TN counties (Grainger, Knox, and Roane) (Etnier et al. 1998), and *Rhyacophila appalachia*, which is known from the Appalachian Mountains of KY, NC, SC, TN, and VA. We are proposing state threatened status for 33 species and special concern status for the remaining 32 species. We acknowledge the preliminary nature of our proposed designations and the lack of detailed distributional information for some species, but we believe formulation of such a list is a reasonable step toward conservation of the group in Kentucky. It is our hope that future revisions to Kentucky's Comprehensive Wildlife Conservation Strategy (KDFWR 2010) will include caddisflies as a group of conservation interest.

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Characteristics and Environmental Problems of a Eutrophic, Seasonally-stratified Lake, Wilgreen Lake, Madison County, Kentucky

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ABSTRACT

Wilgreen Lake (Madison County, Kentucky) is listed as “nutrient impaired” by the United States Environmental Protection Agency and Commonwealth of Kentucky, and it also experiences high fecal microbe counts that restricts its use. The lake is a typical eutrophic lake, experiencing anoxia and dysoxia in its waters during summer stratification. Human activities in the watershed contribute additional nutrients to the lake that may exacerbate periods of anoxia, so knowing the sources of anthropogenic nutrient inputs to the lake would aid in developing best practices for development of lake shore areas and the watershed. Possible sources include residential fertilizers, cattle waste, and human sewage. High nutrient concentrations within surface waters generally occur only proximal to septic system clusters in the upper reaches of Taylor Fork. Bovine and human fecal microbes enter the lake causing periodic high fecal microbe counts, and are likewise restricted to shallow water areas especially after rain events. The areal distribution of high nutrient and fecal microbe values implicate septic systems as the most likely source of these pollutants, but runoff from pastureland must also contribute nutrients and fecal material. We plan to use additional tracing methods in the future to determine the main sources of nutrients and fecal microbes.

KEY WORDS: Eutrophication, nutrient, ammonium, phosphorus, fecal microbe, source tracking

INTRODUCTION

Wilgreen Lake is a eutrophic lake in Madison County formed by damming Taylor Fork (Figure 1), which ultimately feeds Silver Creek and the Kentucky River. The lake's watershed is relatively small (~41 km², ~16 mi²) but is characterized by a variety of land uses that affect the lake ecosystem and impact its water quality. Wilgreen Lake is nutrient “impaired” according to the Environmental Protection Agency (2012) and by the 303(d) listing by Kentucky. The lake provides only non-swimming, recreational use because of episodic, high fecal microbe counts (U.S. EPA 2004a); it is not a source of drinking water. Wilgreen is subject to nutrient loading by livestock production, runoff from developed areas, and septic systems (U.S. EPA 2010). There is no large-scale farming in the watershed. New housing developments

around the lake shore and in proximal areas of the drainage basin have added additional septic systems that ultimately drain into the lake. There is concern that continued and increased delivery of nutrients to the lake may degrade its water quality as eutrophication continues and perhaps accelerates.

We have studied the lake over a period of three years (2006, 2007, and 2008) to make a general assessment of water quality, and ultimately to identify the major nutrient sources that cause eutrophication of Wilgreen Lake. To do so we have monitored the physical and chemical characteristics of the lake (Jolly and Borowski 2007; Hunter and Borowski 2008; Aguiar 2009; Borowski et al. 2009; Stockwell and Borowski 2008), tested for pesticide pollution, and measured fecal microbe concentrations (Borowski and Albright 2007; Aguiar 2009). An advantage to such a concerted effort is to provide a baseline for recognizing changes in eutrophication as the watershed develops and as remediation steps are implemented.

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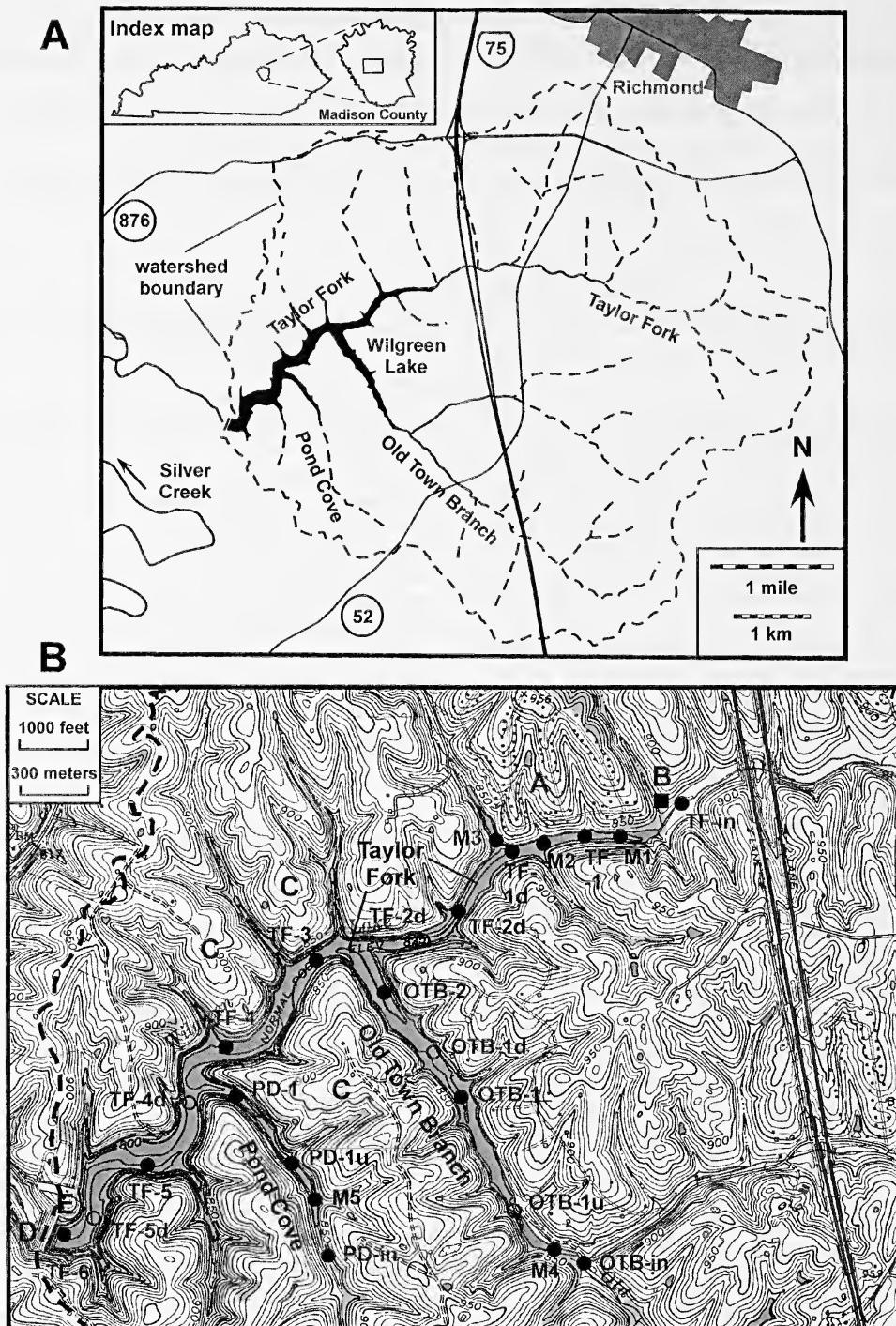


Figure 1. (A) Map of the Wilgreen Lake watershed, Madison County, Kentucky. Note the location of the main streams entering the lake: Taylor Fork, Old Town Branch, and Pond Cove. Also note downtown Richmond, which also extends to the south, east, and west of gray footprint. (B) Topographic map of Wilgreen Lake with stations and surrounding features. Water samples were taken regularly at stations denoted by filled circles; temperature, oxygen concentration, pH, and conductivity were measured at all stations but those with open circles were not regularly

Nature of the Drainage Basin

The watershed is developed within Upper Ordovician limestones, formally designated as the Garrard Siltstone, Calloway Creek Formation, and the Ashlock, Drakes, and Crab Orchard formations (Green 1966; Cressman and Peterson 1986). These units contain interlayered limestone muds, fossiliferous limestone, and siltstone. Numerous sinkholes occur in the drainage basin within the Crab Orchard Formation (Green 1966), and are especially evident in pastures adjacent to the lake (see map in Aguiar 2009) where they are not covered by forest or disguised by human activity. Thus, they likely also occur under developments adjacent to the lake, and at least one sinkhole occurs in Deacon Hills subdivision, about 135 meters from Wilgreen and it is also adjacent to an overland drainage runnel. Although most of our discussions will focus on overland runoff, we realize that groundwater also enters the lake; moreover, groundwater may flow quickly through subsurface karst conduits into the lake, perhaps even from outside the overland drainage basin. Karst conduits may therefore funnel nutrient- and microbe-rich effluent from pastureland, septic systems, and other sources directly into Wilgreen Lake. Unfortunately, it is beyond the scope and abilities of this study to assess the role and nature of these plausible karstic inputs.

Wilgreen Lake is fed by two major streams, Taylor Fork and Old Town Branch, and by several intermittent minor streams (Figure 1). Both of the principal tributaries experience seasonally low flow and during droughts like that of 2007 can fail to flow into the lake. These two watersheds drain land with very different uses. Taylor Fork begins in the urban and industrial areas of southeast Richmond, then winds its way through neighborhoods served by the Richmond sewage system. Only near its inflow to the lake does the drainage basin recover runoff from pastureland and sparse developments on septic systems. A sewage

pumping station occurs approximately 80 meters from the inflow point, and this facility can release raw sewage into the shallow portions of the lake during flooding episodes. We will see that the existence of this pumping station complicates source interpretations for fecal microbes. In the shallowest portion of the lake formed by Taylor Fork, minor runoff occurs from the north and south. The southerly drainage comes from pastureland and wooded bottomland; the northerly drainage comes from the neighborhood of Deacon Hills and Idylwild. This development is characterized by ~240 households utilizing septic systems with as little as half-acre spacing. During the spring and summer, fetid odors near the lake are common in the subdivision indicating leakage of septic systems above ground with the potential of direct runoff into Wilgreen. We suspect this development of being a significant and chronic source for nutrients and fecal microbes to the lake from overland runoff and groundwater seepage; hence, the reason for closely-spaced sampling stations in the upper reaches of lake at Taylor Fork (Figure 1).

Old Town Branch is a very different drainage system passing through mostly widely-spaced households on septic systems and pastureland (Figure 1). Upstream, the source area of the northerly fork of Old Town Branch drains a small industrial area and a high-traffic, two-lane highway (52) before entering a mixture of suburbia and pastureland. The southerly fork covers a larger drainage area covered by widely-spaced dwellings and wooded bottomland. The confluence of the forks occurs only 80 meters from the lake waters. The east shore of lake portion of Old Town Branch is lined by widely-spaced households on septic systems that occur upgradient on relatively steep slopes that lead directly to the lake; the west side of the flooded stream channel is characterized by mostly pastureland at present.

Once the two major streams join, they form the trunk of Wilgreen Lake, which receives water from direct runoff, groundwater, and

←

sampled for water. Letters on the map show pertinent information: (A) developments of Deacon Hills and Idylwild that are served by septic systems; (B) sewage pumping station on Taylor Fork; (C) areas of new developments along the lake served by septic systems; (D) dam; (E) boat dock. Map is from the 7.5 minute quadrangle series (1:24,000), Richmond South quadrangle (photo-revised in 1987), United States Geological Survey.

the small creek of Pond Cove (Figure 1; designated PD). The north side of the lake contains pastureland near the confluence with Old Town Branch, but downstream ~160 new homes (built after our base map, Figure 1B) have been constructed on the ridgeline and slopes overlooking Wilgreen. These homes also have septic systems with ~half-acre spacing. The south side of the lake trunk is occupied mostly by pastureland although an unoccupied development, cleared before 2006, occurs on the divide between Old Town Branch and Pond Cove. The development contains ~200 lots that will be served by septic systems. Near the dam, a floating boat dock provides recreational access to the lake.

Pesticide Pollution

To assess pesticide pollution within Wilgreen we assay for 2,4-dichlorophenoxyacetic acid (subsequently referred to 2,4-D), s-metolachlor, atrazine, and alachlor. 2,4-D is a chlorinated phenoxy compound that is used to control many types of broadleaf weeds; the U.S. EPA has determined that concentrations greater than 290 mg/L (290,000 µg/L) are potentially harmful to aquatic organisms (U.S. EPA 2005). s-metolachlor is used to control certain broadleaf and annual grassy weeds; the U.S. EPA has determined that concentrations greater than 0.78 mg/L (780 µg/L) are potentially toxic to aquatic animals (U.S. EPA 1995). Atrazine is widely used to control broadleaf weeds and some grassy weeds; the U.S. EPA has determined that concentrations greater than 37 µg/L are potentially toxic to aquatic organisms (U.S. EPA 2006). Alachlor is used to control broadleaf and grassy weeds on a number of crops; the U.S. EPA has determined that concentrations greater than 0.1 to 0.2 mg/L (100 to 200 µg/L) are potentially toxic to aquatic animals (U.S. EPA 1998).

Nutrient Pollution

Increased nutrient (ammonia, nitrate, phosphate) levels within natural waters have become an acute and widespread problem in the natural waters of the United States (Dubrovsky et al. 2010). Although eutrophification does occur naturally, most nutrient pollution arises because of use of fertilizers,

manure produced by farm animals, atmospheric deposition, and human sewage. Localities unaffected by human activities are few, but Dubrovsky et al. (2010) have determined background, non-anthropogenic concentrations for ammonia, nitrate, and phosphate of 0.025, 0.24, and 0.01 mg/L, respectively, in streams.

The U.S. EPA and other governmental agencies have also determined acute and chronic criteria for nutrient levels within natural waters. Acute instances occur when concentrations of contaminants spike over a period of days; chronic conditions occur when concentrations are not as high but occur for longer time periods. The rationale for this rating system is that organisms have different tolerances for short-term versus long-term exposure. In the case of ammonia, acute and chronic criteria are dependent on pH, temperature, and whether salmonid fish and/or fish in early life stages are present in the aquatic system. For freshwater with pH of 8.0, the threshold values for acute criteria are 5.62 and 8.40 mg/L nitrogen, respectively, or 7.2 and 10.8 mg/L NH₄; chronic criteria for a pH of 8.0 at 16°C are identical for cases with and without fish in early life stages at 2.21 mg/L nitrogen, or 2.8 mg/L NH₄ (U.S. EPA 2009). The Minnesota Pollution Control Agency, using methods outlined by the U.S. EPA (1985), determined that nitrate standards for cold freshwater with and without lake trout should be 3.1 and 4.9 mg/L N, respectively, or 13.7 and 21.7 mg/L NO₃. Total phosphate in lakes should not exceed 0.05 mg/L whereas stream levels should be below 0.1 mg/L (MPCA 2010).

Nutrients in lake systems provide for increased growth of phytoplankton and macroalgae because nitrogen and/or phosphorus can be limiting factors in plankton growth. Eutrophification can be a natural process because lakes tend to accumulate nutrients, but it is usually intensified by anthropogenic addition of nutrients (Wetzel 1975). Added primary production can affect water quality by increasing the amount of biomass available for decomposition. Decomposition in deeper lake waters and sediments consumes oxygen leading to anoxia in bottom waters. Anoxia decreases living space for oxygen-utilizing organisms within the lake ecosystems. In

extreme cases, eutrophication can lead to “dead” lakes, dominated by anaerobic microbes and excluding oxygen-utilizing animals. A host of other problems like harmful algal blooms, foul-smelling and tasting water, decrease in water clarity, and fish kills are also attributes of excessive eutrophication (Dubreovsky *et al.* 2010).

Possible Nutrient Sources

We suspect that leachate from the septic systems of Deacon Hills and Idylwild housing developments (Figure 1B) is a major contributor to eutrophication in Wilgreen Lake. Newer developments with approximately 160 collective households have been built in the watershed adjacent to Wilgreen Lake, and as their septic systems age there is possibility of additional septic effluent entering the lake through groundwater. Indeed, if situated on karstic conduits septic fluids may be already entering the lake from these newer developments. In the future, these new developments and new building on undeveloped lands may increase the nutrient load to the lake.

Other possible nutrient sources include stream input draining pastureland as well as direct runoff from pastureland into the lake, fertilizer run-off, and sewage from other sources entering the lake. Septic systems are used in developments ringing the lake and it can also contribute leachate to either surface or subsurface waters. Knowledge of the principal source or sources is essential in creating policies and strategies to abate further nutrient loading. For example, Madison County is encouraging the installation of fences along the lake border that prevent cattle from entering lake waters and directly depositing body wastes into the lake. If septic systems are the major source of nutrients and fecal matter, these proposed remediation steps will not improve the lake’s water quality. Targeted remediation should be much more effective.

Fecal Microbe Pollution

Wilgreen Lake is designated as a recreational lake, suitable only for secondary human contact (non-swimming or recreational contact) because of high fecal microbe counts (U.S. EPA 2004a). The U.S. EPA first set standards for coastal recreational waters in

1986, using multiple water samples (a calculated geometric mean) in order to set safe levels for a variety of recreational uses (U.S. EPA 1986). Because of the difficulty and expense in procuring and processing multiple samples, the U.S. EPA sought to create standards based on single samples for state waters (EPA 2004a). Thus, standards for single-sample analyses for *Escherichia coli* have been established by the EPA (2004b) and are also accepted by Kentucky state government in the form of Kentucky Administration Regulations (KAR 401 5:031). The U.S. EPA designations for surface, recreational waters for use by humans are: *bathing acceptable* (<235 cfu/100 mL), *recreational use only* (236–574 cfu/100 mL), and *no human contact recommended* (>575 cfu/100 mL). There are also sub-designations for recreational use that are largely dependent on systematic, multiple samples (EPA 2004b), but we analyze our data in the light of the broader designations. Standards for total coliform counts have been established by Kentucky (KAR 401 5:031); the U.S. EPA has not established values for total coliform.

Total coliform and *E. coli* counts have been used to access water quality, but experience has shown that *E. coli* counts are more reliable as indicators of fecal contamination, whereas total coliform counts have multiple sources other than fecal material (U.S. EPA 1986; Dick and Field 2004). Fecal material entering the lake is also a source for nutrients so that *E. coli* counts also represent addition of nutrients.

METHODS

Water Sampling

Twenty-four sampling stations have been established on Wilgreen to document the areal and vertical variability of physical and chemical parameters (Figure 1). Water samples were collected at 1-meter depth increments and were used to measure nutrient concentration; samples at depth were collected with a Van Dorn sampler. Each sample was filtered on location through a 0.45 micron (μm) syringe filter and split into three subsamples for analysis of ammonium, nitrate, and phosphate (Figure 2). These sub-samples were acidified with H_2SO_4 to bring the sample

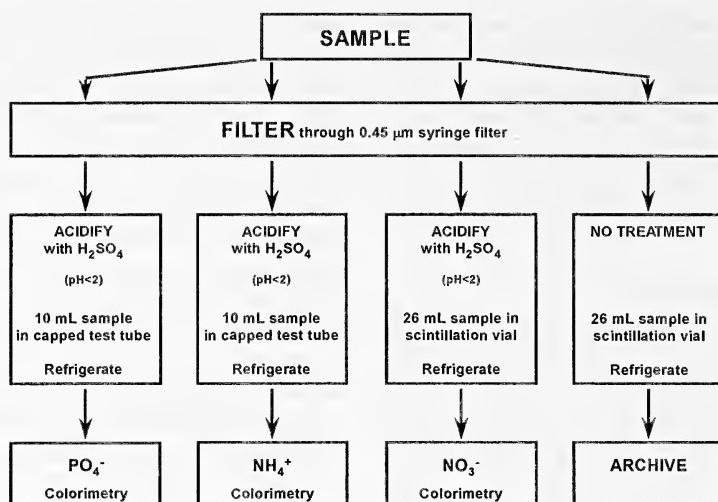


Figure 2. Diagram showing the treatment and use of water samples taken from Wilgreen Lake.

to pH of ≤ 2 in order to prevent sample degradation (Clesceri et al. 1998). Another 26-mL subsample was untreated and used as an archive sample. Samples were then placed in a cooler on ice, and ultimately transported to the laboratory, where they were refrigerated. Nutrient measurements were conducted within 30 days as specified by Clesceri et al., 1998.

Water samples for pesticide analyses were treated differently. At shallow stations, we collected only a near-surface sample whereas at deeper stations we also collected a sample from deeper, anoxic waters using a Van Dorn sampler. Samples were unfiltered, not acidified, and collected in pre-washed, amber bottles. Analyses occurred immediately after sampling.

Physical Properties

We use an YSI multi-parameter probe (model 556 MPS) to simultaneously measure temperature, conductivity, pH, and oxygen concentration. Probe calibration was completed daily with appropriate reference solutions (YSI User Handbook 2002); pH calibration used the three point method. Probe measurements of oxygen at extremely low levels (<0.09 mg/L) seem less reliable; we report oxygen concentration as zero (anoxic) for water samples containing hydrogen sulfide (H_2S) as identified by smell.

Pesticides

For measurement of 2,4-D, s-metolachlor, atrazine, and alachlor we used immunoassay

kits supplied by Beacon Analytical Systems, Inc. We tested for 2,4-D and S-metolachlor using tube versions of the kits in the first round of testing in October of 2007. The kits supplied test tubes that were coated with polyclonal antibodies that bind the pesticide, an enzyme conjugate of the pesticide that will also bind to the antibodies coated on the tubes, substrate to react with the enzyme conjugate to produce a colored solution that absorbs at 450 nm, and pesticide standards needed in the analysis. Water samples, spikes, and standards were added to the tubes, followed by the addition of the enzyme conjugate. The pesticide in the samples will compete with the enzyme conjugate for binding sites on the antibodies. After a prescribed incubation period, unbound molecules were washed away with distilled water. A colorless substrate solution was then added to each test tube. The enzyme conjugate molecules that are bound to the antibodies convert the substrate to a blue compound. A darker solution indicated a lower concentration of pesticide because more antibodies are bound with the enzyme conjugate, which reacts with the substrate to produce the colored solution; the intensity of the color developed in the solutions during the analysis is inversely proportional to the pesticide concentration. Kit instructions were followed for each pesticide and the absorbances of the solutions in the tubes were measured using a spectrometer.

Table 1. Parameters for pesticide analysis.

Pesticide method	Standards range ($\mu\text{g/L}$)	Quality control spike levels ($\mu\text{g/L}$ - % recovery)
2,4-D tube kit	2–100	10–150%
2,4-D plate kit	2–200	2–65%
s-melolachlor tube kit	0.05–3.0	20–101%
		0.6–98%
		0.6–83%
		0.6–118%
s-melolachlor plate kit	0.05–4.0	0.05–53%
		1.0–120%
atrazine plate kit	0.05–5.0	0.3–93%
alachlor plate kit	0.10–0.75	0.1–99%

More extensive testing for 2,4-D, S-metolachlor, atrazine, and alachlor was accomplished in May of 2008 using plate immunoassay kits (Beacon Analytical Systems, Inc.) The kits were analogous to the tube kits described above except that 96-well plates coated with the appropriate antibodies were supplied instead of coated test tubes. The reading of the absorbances in the 96-well plates was accomplished using a Bioteck Synergy II plate reader.

The concentration range of the standards used for each immunoassay along with the spike recoveries appear in Table 1. Samples whose concentrations fall below the lowest standard are reported as less than that standard concentration. Thus, the analyte can be either absent or either present at immeasurable levels if its concentration is less than the lowest standard concentration.

Ammonium Concentration

To measure ammonium concentration, we used the Berthelot reaction (phenol hypochlorite method) as described by Solorzano (1969) (see also Gieskes *et al.* 1991; Eaton *et al.* 2005, Method 4500-NH₃ F) using colorimetry and a spectrophotometer. The method is sensitive and specific to ammonium (NH₄⁺) and ammonia (NH₃) (Eaton *et al.* 2005). Standards [0.0, 0.5, 1.0, 2.5, 5.0, and 12.5 mg/L NH₄] were used to create a linear standard curve, and two spiked samples [0.5, 1.0 mg/L] were prepared and measured with each batch of lake samples; standards and samples were treated identically. Standard curves have r² values (correlation coefficient) greater than 0.994. Detection limits are <0.1 mg/L (Method 4500-NH₃ F, Eaton *et al.* 1995); we report values to the nearest 0.1 mg/L and values <0.1 mg/L are reported as zero.

Nitrate Concentration

We used cadmium reduction, another colorimetric method (Eaton *et al.* 2005, Method 4500-NO₃ E), to measure nitrate using Hach NitraVer 5 reagent packets (Hach 1986). Because we acidified our samples, we actually measured nitrite (NO₂⁻) and nitrate (NO₃⁻) but report the values as NO₃. Nitrate data occur only for waters sampled during the 2008 field season. Standards [0.0, 0.5, 1.0, 2.5, 5.0, and 12.5 mg/L N-NO₃] yielded standard curves with r² values greater than 0.994. The detection limit for nitrate is theoretically 10 $\mu\text{g}/\text{L}$ (~44 $\mu\text{g}/\text{L}$ N-NO₃, Method 4500-NO₃ E, Eaton *et al.* 1995) but our experience shows reduced confidence in concentrations <0.1 mg/L (100 $\mu\text{g}/\text{L}$). Consequently, we report values to the nearest 0.1 mg/L and values <0.1 mg/L are reported as zero.

Phosphate Concentration

Phosphate (PO₄³⁻) concentration of the lake water was measured using the ascorbic acid, colorimetric method (Strickland and Parsons 1968; Gieskes *et al.* 1991; see also Eaton *et al.* 2005, Method 4500-P E). Because we filtered our samples, the method measures only dissolved orthophosphate. Standards [0.0, 0.25, 0.5, 1.0, and 2.5 mg/L PO₄] were prepared with each batch of lake samples to establish a linear standard curve; typical r² values were greater than 0.996. Samples outside the linear portion of the curve (>2.5 mg/L) were re-analyzed using a dilution factor of 2 and in one case (station M1 – September 2007) it was necessary to use a dilution factor of 4. The detection limit is theoretically 10 $\mu\text{g}/\text{L}$ (Method 4500-P E, Eaton *et al.* 1995) but our experience shows reduced confidence in concentrations <0.1 mg/L

L (100 µg/L). Consequently, we report values to the nearest 0.1 mg/L and values <0.1 mg/L are reported as zero.

Fecal Microbes

We sampled the surface waters at selected stations across the lake to assess bacterial abundance on two instances in 2006 and 4 times in 2007, sampling during normal and dry conditions during the summer field season. Sampling was concentrated in the proximal, shallow-water areas of Old Town Branch, Taylor Fork, and Pond Cove but we also sampled at the deeper-water stations along the trunk of Taylor Fork.

We determined the distribution and abundance of *Escherichia coli* and total coliform bacteria within Wilgreen Lake using the rapid assay method developed by IDEXX (2006). Unfortunately, cost and equipment limitations prevented using other methods involving determination of atypical colonies (e.g., Brion and Mao 2000) and other potential indicators such as human epicoprostanol and fecal load indicators (Black et al. 2007). The IDEXX method uses *Colisure®* materials now accepted by the EPA, American Water Works Association, American Public Health Association, and Water Environment Federation as an established method in quantifying the occurrence and abundance of these microbes (Method 9223, Eaton et al. 2005). The method gives an approximate count of microbe abundance in terms of colony-forming units (cfu) per 100 mL, as estimated from statistical methods that determines the most probable number (MPN) of colonies (IDEXX 2006).

Samples were taken and analyzed over a period of two days. The procedure requires the collection of 100-mL water samples in sterile bottles according to standard collection methods (Method 9060, Eaton et al. 2005). Water samples were stored on ice in the field and transported to the laboratory within six hours (IDEXX 2006; Method 9060, Eaton et al. 2005). At the lab, processing began immediately when pre-packaged *Colisure®* indicator reagent was added to samples and thoroughly mixed. Samples are then poured into a IDEXX Quanti-Tray®, sealed using a Quanti-Tray Sealer®, and incubated for 24 hours at

35°C. After incubation, total coliform was measured by counting the number of large and small wells that turned red/magenta. *E. coli* abundance was determined by counting the number of wells that fluoresce purple under UV light. The raw total coliform and the *E. coli* counts are then converted to the most probable number (MPN) of colonies per 100 ml using an IDEXX table. We present and analyze only *E. coli* data because these counts directly indicate fecal contamination.

The IDEXX method can only register a maximum microbial count of 2419 cfu/100 mL without sample dilution, so in some of these cases we diluted samples to obtain absolute counts, especially those samples from 30 June 2007. Subsequent to 30 June, some stations have replicate samples that were prepared using a 1:4 dilution as described by Brazos River Authority (2003), and we also tested a dilution factor of 1:10 to compare counts between undiluted and diluted samples. In most cases, only a slight discrepancy (<10% for *E. coli*) was noted (Aguiar 2009).

RESULTS

Physical and Chemical Properties

Wilgreen Lake is a typical eutrophic lake showing thermal stratification, which affects other physical and chemical parameters (Figure 3). Lake waters behave uniformly throughout the field season at the deep water stations along the trunk of Taylor Fork (stations TF-6 to TF-2), and extending to stations OTB-2 and PD-1 within the tributaries (Figure 1). Shallow-water stations near stream entries do not stratify and tend to exhibit more variability, responding to inflow from normal runoff and to rain events within the watershed. Wide variations in temperature and conductivity (0.307 to 0.900 mS/cm²) occur dependent on inflow conditions with oxygen generally high (up to 17.72 mg/L) and pH ranging from 7.24 to 8.85.

The following description of lake properties relates mostly to deeper water stations of the lake that stratify and that contain the preponderance of the lake's volume. The upper layer is warm (typically over 20°C during the summer and Fall), the lower layer is cool (about 8 to 10°C) with a transitional zone

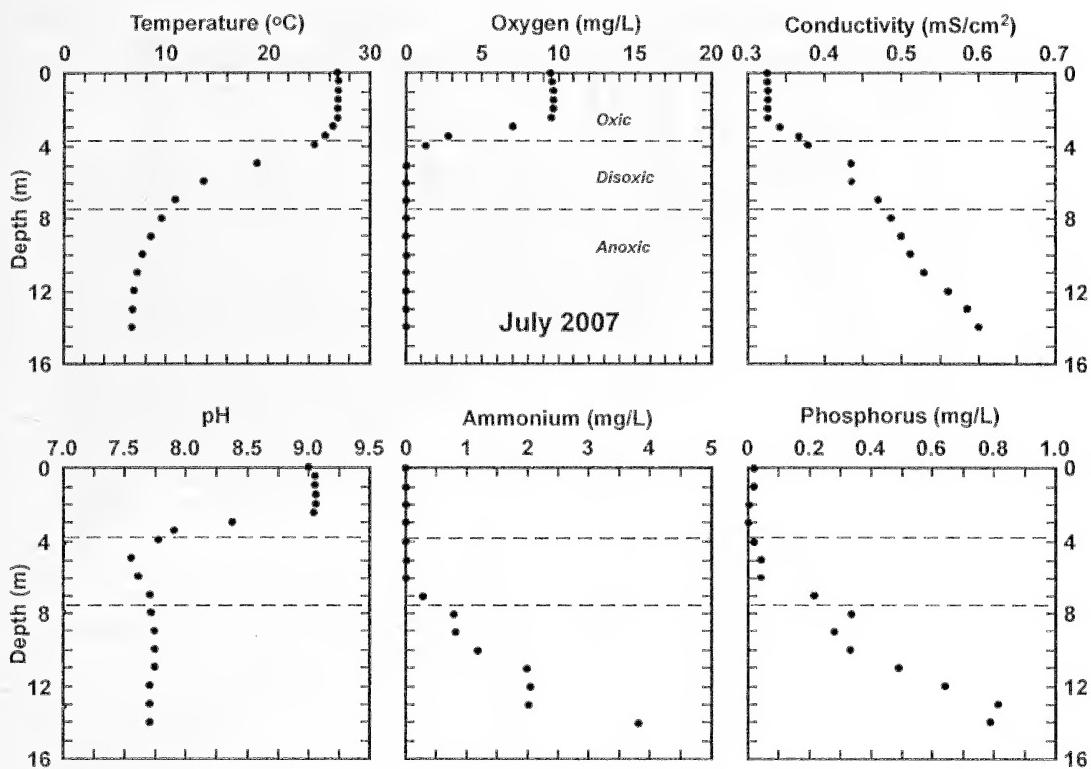


Figure 3. Typical profiles of selected parameters that occur during stratification in the summer. Measurements and samples were taken on 23 July 2007 at Station TF-6, the deepest sampling station (see Figure 1). Shown are profiles of temperature (degrees Centigrade, °C), oxygen concentration (milligrams/Liter, mg/L), conductivity (milliSiemens/square centimeter, mS/cm²), pH, nitrogen concentration as ammonium (NH₄), and phosphorus concentration as phosphate (PO₄). Dashed horizontal lines refer to oxygen concentrations at either oxic (>2 mg/L), dysoxic (>0 to 2 mg/L), or anoxic levels (0 mg/L) within the water column. Data from Hunter and Borowski (2008); Aguiar (2009).

between containing the thermocline. During stratification, oxygen, conductivity, pH, and nutrient concentrations are very different in the upper versus lower layers (Figure 3). Oxygen concentration responds to stratification, water temperature, the balance between photosynthesis and respiration in the upper layer, and the rate of decomposition in deeper waters of the lake (e.g., Wetzel 1975). Oxygen levels are higher in the upper lake layer and are maintained by addition of oxygen from photosynthesis and by addition from the atmosphere, whereas deeper waters beneath quickly lose oxygen via microbial decomposition of organic matter to become dysoxic (O₂ between 0 and 2 mg/L) or anoxic (0 mg/L) (e.g., Wetzel 1975); anoxic waters smell strongly of hydrogen sulfide (H₂S). Conductivity is typically lower in the upper layer but increases markedly below the thermocline as dissolved ions are added by decomposition

reactions. The upper layer generally has more alkaline pH (8.2–9.1), likely because slightly acidic rainwater is buffered by the limestone bedrock of the drainage basin, but deeper waters become more acidic concomitant with net decomposition of organic matter, which adds increasing amounts of dissolved carbon dioxide (CO₂) and organic acids (e.g., Wetzel 1975). Nutrient concentrations (nitrogen as ammonium, NH₄, and as nitrate, NO₃; phosphorus as phosphate, PO₄) are generally lowest in the upper layer where uptake by phytoplankton and algae occurs, and are highest in the lower layer as nitrogen and phosphorus are liberated by organic matter decomposition in the water column and sediments (Figure 3). All nutrients follow this same basic pattern with depth at the trunk stations. Anthropogenic sources of nutrients to Wilgreen Lake affect its water quality and we will focus on their sources below.

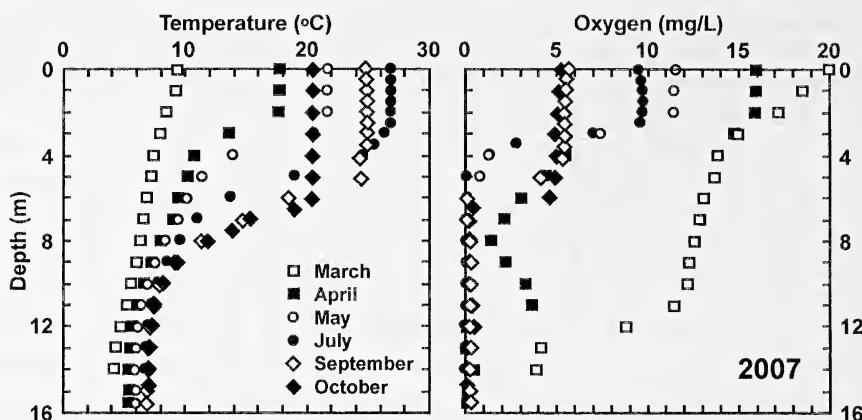


Figure 4. Seasonal variation in temperature and oxygen concentration as a function of water depth. Data represent profiles at Station TF-6 (see Figure 1) during the field season of 2007. Symbols are keyed to sampling on 13 March, 27 April, 17 May, 23 July, 12 September, and 12 October. Data from Hunter and Borowski (2008); Aguiar (2009).

Wilgreen Lake goes through a typical seasonal cycle for a eutrophic lake, showing stratification in the spring and summer with fall turnover occurring sometime in October or shortly thereafter, with stratification developing again in March (Figure 4). Deep waters approach 4°C at their coolest observed temperature whereas surface water fluctuates from freezing to slightly over 30°C; the highest observed temperature was 32.9°C occurring at Station TF-4 in August 2006 and 2007 (Aguiar 2009; Jolly and Borowski 2007; Hunter and Borowski 2008). Temperature data from 2007 shows that surface waters begin warming in March with stratification established by late April with the upper layer being about 2 meters thick (Figure 4). The lake remains stratified through the summer and into the fall, but the thermocline deepens from 2 meters in April, to 3 m in May, remaining there with some fluctuation through June, July and August with temperature of the upper mixed layer increasing from about 22 to 26, 27, and 30°C, respectively. Surface lake waters then begin to cool from 25° to 20°C in September and October as the thermocline deepens to 5 and 6 m respectively. Temperature and corresponding density differences between the upper and lower layers decline further and the mixed layer deepens until Fall turnover occurs; our observations do not witness the final collapse of summer stratification within the lake.

Dissolved oxygen concentrations also change seasonally (Figure 4) responding to water

temperature, stratification, the balance between photosynthesis and respiration in the upper layer, and the rate of decomposition in deeper waters of the lake. In surface waters, oxygen concentration is at its maximum in the early spring (~20 mg/L in March 2007) and declines progressively into the summer with minimum values of ~5 mg/L occurring in September and October. Stratification profoundly influences oxygen distribution with depth as water remains well oxygenated in the upper layer and but is oxygen-poor below the thermocline. In 2007, March is the only observed month when deep waters are well-oxygenated, otherwise oxygen concentration below the thermocline is generally <1 mg/L. Cross sections of the lake (Figures 5, 6) show that the preponderant volume of Wilgreen is either dysoxic or anoxic during stratification. The thickness of the dysoxic layer is generally about 2 to 4 meters whereas the bottom-most 6 to 10 meters of the lake are anoxic through summer stratification at the deepest stations (TF 6 though TF-4), thinning upstream toward shallower stations.

Lake Bathymetry

Wilgreen lake is a dammed stream and a cross section of the lake shows the nature of the former stream topography (Figure 5). The lake is deepest at Station TF-6 just upstream from the earthen dam. The lake progressively shallows upstream along the trunk of the lake with a marked decrease in depth in the transition from stations TF-3 to TF-2d.

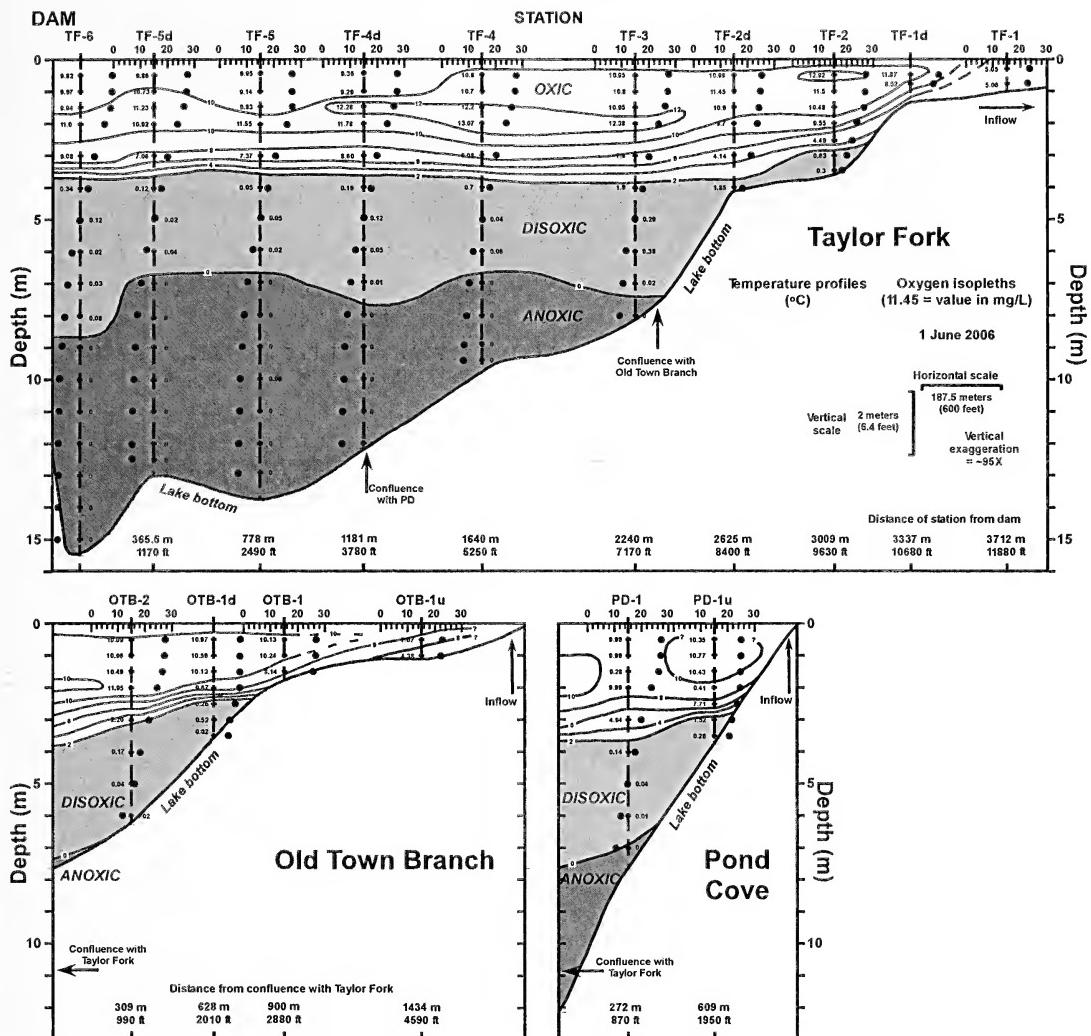


Figure 5. Cross section of Wilgreen lake along the main trunk of the lake (Taylor Fork) and its tributaries, Old Town Branch and Pond Cove (see Figure 1), showing graphed temperature data ($^{\circ}\text{C}$, filled circles) and oxygen concentration isopleths for data measured on 1 June 2006. Oxygen values in units of milligrams per liter (mg/L) for each station are shown next to the corresponding depth; the contour interval for oxygen isopleths is 2 mg/L. Shading refers to oxygen concentrations at either oxic ($>2 \text{ mg/L}$; no shading), dysoxic (>0 to 2 mg/L ; light gray), or anoxic levels (0 mg/L ; darker gray) within the water column. The entire lake segment is shown in the cross sections of Old Town Branch and Pond Cove, however a small portion of shallowest portion of Taylor Fork is not shown. Note the horizontal and vertical scales and the vertical exaggeration of ~ 95 times. Note also the locations of the confluences on each cross section, and the distances from either the dam (Taylor Fork) or a confluence (Old Town Branch, Pond Cove) shown at the bottom of each cross section panel. Data from Jolly and Borowski (2007).

Moving up Old Town Branch, the lake becomes noticeably shallower leaving the trunk of lake (station OTB-2) moving upstream to stations OTB-1 through M4. Within Pond Cove, Station PD-1 is deep but stations PD-1u and M5 are considerably shallower. At and upstream of stations TF-1d, OTB-1, and PD-1u lake waters are strongly influenced by

inflow from streams and by other local conditions.

Homogeneity of Deep-Water Stations

Wilgreen Lake tends to be physically and chemically homogeneous over most of its areal extent, especially at deep-water locations, which contain the preponderance of its

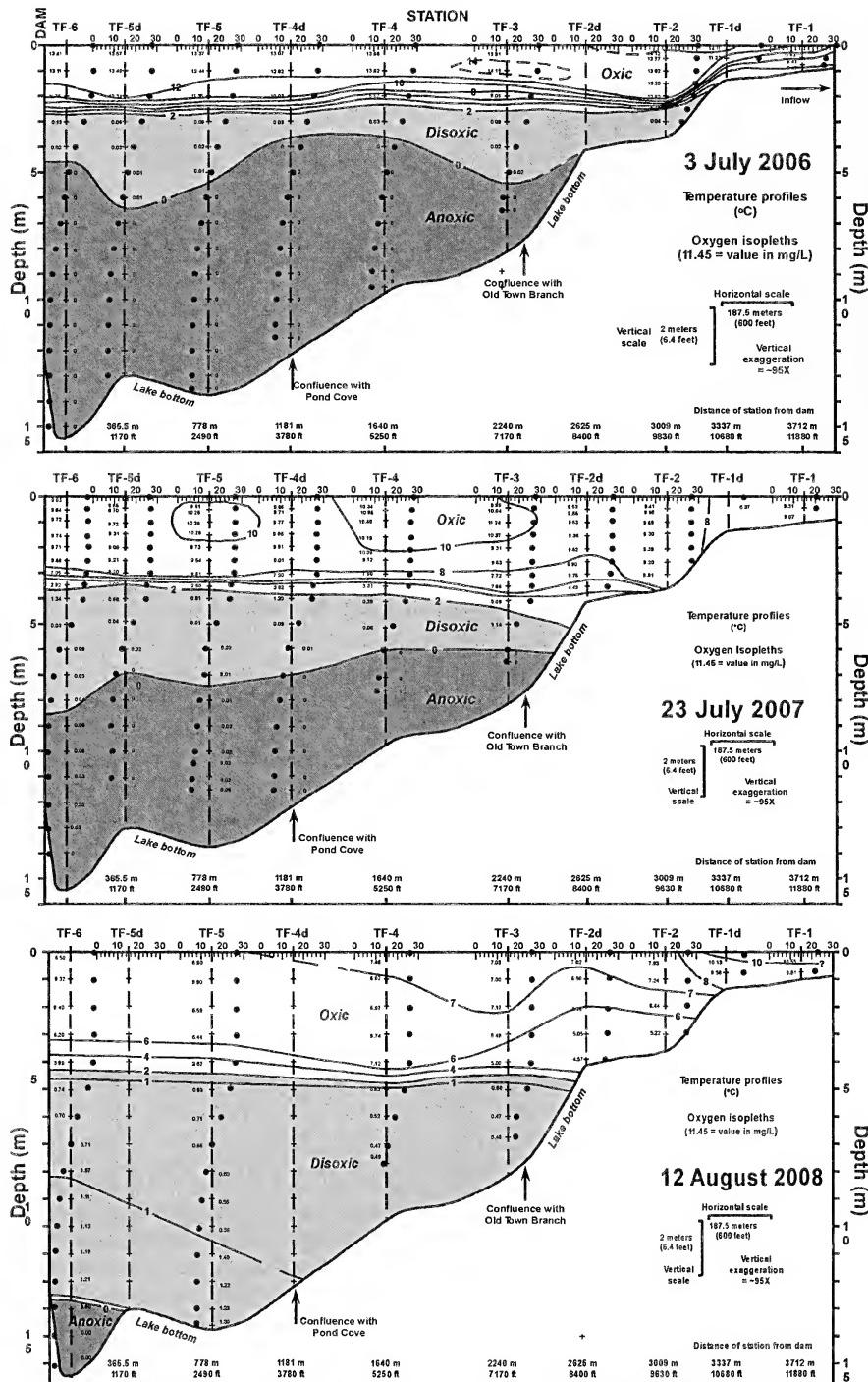


Figure 6. Cross sections of Taylor Fork during maximum stratification during the three successive years of 2006, 2007, and 2008. See caption of Figure 5 for explanations. Data from Jolly and Borowski (2007), Hunter and Borowski (2008), Stockwell and Borowski (2008), and Aguilar (2009).

volume. Figure 5 shows lake cross sections with temperature profiles and oxygen isopleths of the lake along the trunk of Taylor Fork and its tributaries, Old Town Branch and Pond Cove. Temperature data show a thermocline about 3 meters in depth that covers most of the deeper-water stations of the lake. Stratification becomes less pronounced at upstream stations (shallower than TF-2d, OTB-1d, and PD-1u), and where inflowing stream waters have more effect on lake properties. Oxygen concentrations are characteristically also similar across the lake. The cross sections clearly show that oxic waters occur in the upper, warmer layer of the lake and that oxygen decreases with depth to dysoxic and finally anoxic levels. Moreover, oxygen isopleths roughly parallel the lake surface across the deeper waters of the lake highlighting the strong correlation between physical and chemical stratification. Although not shown with any cross sections, nutrient concentrations are also homogeneous across the deeper portions of Wilgreen Lake with generally very low concentration in the uppermost, sunlit layer of the lake and progressively increasing concentration into colder, dysoxic to anoxic waters (Figure 3). Homogeneous nutrient concentrations observed at the deep-water stations of the lake allow us to recognize anomalously high nutrient concentrations in shallow-water portions of the lake for source tracking.

Annual Variation

Although lake parameters are generally uniform across the deep-water portions of the Wilgreen Lake, the lake displays marked differences in its properties from year to year. The lake always stratifies but temperature and oxygen properties change annually in response to solar heating and rainfall. Over the course of this study, the summer months of 2006 and 2007 were very dry but much wetter in 2008; 2006 was the hotter year. Figure 6 shows cross sections along the trunk of the lake during summer stratification from 2006 to 2008 during the height of stratification. The position of the thermocline is similar during each year but the strongest variation is seen in oxygen content of the lake. Oxygen isopleths defining the upper and lower layers of the lake are much closer in 2006 indicating a sharper oxygen gradient positioned at 2.5 m. In 2007,

the oxygen gradient is not as sharp (depth a bit deeper than 3 m), and in 2008 oxygen gradients are diffuser still with the sharpest gradient slightly deeper at 4.5 m. Overall oxygen content mirrors this trend with the collective volume of dysoxic and anoxic water decreasing as the summer season becomes less hot and wetter from 2006 to 2008. The volume of anoxic water is also highest during 2006, overall the hottest and driest summer. Summer 2008 is most atypical with a very small amount of anoxic water hugging the bottom. These significant annual variations will make it difficult to recognize larger trends of nutrient loading that may occur within Wilgreen Lake in the future.

Pesticides

Our pesticide assay results are tabulated in Table 2. Potentially toxic levels of s-metolachlor (780 µg/L), atrazine (37 µg/L), and alachlor (100–200 µg/L) are not observed in any of our samples. All samples have <2 µg/L of 2,4-D, whereas the EPA level for potential toxicity for aquatic organisms is 0.29 µg/L. Thus, we cannot determine if Wilgreen waters contain potentially harmful amounts of 2,4-D. The lack of pesticide residues within our samples is not surprising as little or no commercial agriculture takes place within the Wilgreen drainage basin. In the absence of other evidence, we infer than 2,4-D behaves like the other measured pesticides and conclude that pesticide pollution is apparently not a problem in Wilgreen Lake, so will not discuss these findings further.

Outward Evidence of Eutrophification

Wilgreen Lake is listed as nutrient impaired and there are obvious signs of eutrophification. Large mats of algae exist particularly in the shallow reaches of Taylor Fork and Old Town Branch proximal to suspected nutrient sources. These mats consist of green algae (*Chlorophyta*); the dominant genera are *Oedogonium*, *Desmidium*, and *Microspora*; *Cosmarium*, and *Mougeotia* also occur (R. Creek pers. comm., 13 June 2009).

Overall Nutrient Concentration

Most samples contain nutrient concentrations higher than systems without human

Table 2. Pesticide measurements of 2,4-dichlorophenoxyacetic acid (2,4-D), s-metolachlor, atrazine, and alachlor during two different field seasons at Wilgreen Lake.

Station	October 2007			May 2008					
	Depth (m)	2,4-D ($\mu\text{g/L}$) ²	s-Metolachlor ($\mu\text{g/L}$) ²	Station	Depth (m)	2,4-D ($\mu\text{g/L}$) ²	s-Metolachlor ($\mu\text{g/L}$) ²	Atrazine ($\mu\text{g/L}$) ²	Alachlor ($\mu\text{g/L}$) ²
TF in ¹	—	<2	<0.05	TF in ¹	—	<2	<0.05	—	—
M1	0	<2	<0.05	M1	0	<2	<0.05	<0.1	<0.1
TF-1	2	<2	<0.05	TF-1	0	<2	<0.05	<0.1	<0.1
	2	<2	<0.05		—	—	—	—	—
M2	—	—	—	M2	0	<2	<0.05	<0.1	<0.1
TF-1d	—	—	—	TF-1d	0	<2	<0.05	<0.1	<0.1
M3	—	—	—	M3	0	<2	<0.05	<0.1	<0.1
TF-2	—	—	—	TF-2	—	—	—	—	—
TF-2d	—	—	—	TF-2d	—	—	—	—	—
TF-3	0	<2	<0.05	TF-3	0	<2	<0.05	<0.1	<0.1
	4	<2	<0.05		—	—	—	—	—
	4	<2	<0.05		—	—	—	—	—
TF-4	0	<2	<0.05	TF-4	0	<2	<0.05	<0.1	<0.1
	0	<2	<0.05		8	<2	<0.05	<0.1	<0.1
	6	<2	<0.05		—	—	—	—	—
TF-4d	—	—	—	TF-4d	—	—	—	—	—
TF-5	0	<2	<0.05	TF-5	0	<2	<0.05	<0.1	<0.1
	11	<2	<0.05		—	—	—	—	—
TF-5d	—	—	—	TF-5d	—	—	—	—	—
TF-6	0	<2	<0.05	TF-6	0	<2	<0.05	<0.1	<0.1
	13	<2	<0.05		8	<2	<0.05	<0.1	<0.1
OTB in ¹	—	<2	<0.05	OTB in ¹	—	<2	<0.05	<0.1	<0.1
M4	0	<2	<0.05	M4	0	<2	<0.05	<0.1	<0.1
OTB-1u	0	<2	<0.05	OTB-1u	0	<2	<0.05	<0.1	<0.1
OTB-1	1	<2	<0.05	OTB-1	0	<2	<0.05	<0.1	<0.1
OTB-1d	—	—	—	OTB-1d	—	—	—	—	—
OTB-2	0	<2	<0.05	OTB-2	0	<2	<0.05	<0.1	<0.1
	0	<2	<0.05		6	<2	<0.05	<0.1	<0.1
	4	<2	<0.05		—	—	—	—	—
	4	<2	<0.05		—	—	—	—	—
PD-in ¹	—	—	—	PD-in ¹	—	<2	<0.05	<0.1	<0.1
M5	0	<2	<0.05	M5	0	<2	<0.05	<0.1	<0.1
PD-1u	1	<2	<0.05	PD-1u	0	<2	<0.05	<0.1	<0.1
PD-1	0	<2	<0.05	PD-1	0	<2	<0.05	<0.1	<0.1
	6	<2	<0.05		7	<2	<0.05	<0.1	<0.1

¹ Stream samples taken without regard for depth.

² Analyte concentrations are reported as below the lowest standard concentration (see Table 1).

influence. The percentage of samples over the natural, background levels for ammonium (0.025 mg/L), nitrate (0.24 mg/L), and phosphate (0.01 mg/L) (Dubravsky et al. 2010) are 39%, 52%, and 68%, respectively. A much smaller percentage of samples shows nutrient values exceeding acute and chronic values for aquatic systems. For ammonium, none and 5.2% of samples exceed the threshold values of 10.8 mg/L and 2.8 mg/L for acute and chronic exposure, respectively (EPA 2009). For nitrate, only 0.4% of all samples exceed the threshold of 13.7 mg/L NO₃ (3.1 mg/L N-NO₃; MPCA 2010), assuming average pH of 8.0 and temperature of 16°C. For phosphate, 36% of samples exceed the threshold value of

0.05 mg/L (MPCA 2010). The location of higher nutrient concentrations, especially those in excess of accepted threshold values, should suggest major nutrient sources.

Nutrient Content of Deeper Waters

Once stratification occurs nutrient concentrations (ammonium, nitrate, phosphate) are almost always higher in lake waters below the thermocline relative to surface water values (Figure 3) (Aguiar 2009). Nutrients are at low concentration in the upper several meters of the lake at deep-water stations until Fall turnover occurs when dissolved nutrients in the deeper layers are injected toward the surface (Aguiar 2009). Average ammonium

and phosphate concentration within the lower layer of the lake during stratification are 1.6 and 0.9 mg/L respectively; measured maximum values were 6.8 mg/L (TF-5, 13 m, September 2006) and 3.9 mg/L (TF-6, 8 m, October 2007), respectively (Aguiar 2009). None of the ammonium values exceed the recommended threshold amount for acute criteria, whereas about 3.9% of deep-water samples (3.2% of all samples) exceed threshold amounts for chronic criteria. For phosphate, 61% of deep-water samples exceed the threshold value (0.05 mg/L PO₄).

Nutrient Concentrations in Surface Waters

For shallow (<2 m) and surface waters, consistently high nutrient concentrations occur only in the shallow portions of the lake, proximal to suspected anthropogenic nutrient sources. For example, Figure 7 shows phosphate concentration in surface waters for 2007. Note that phosphate levels are generally <0.1 mg/L at deep-water stations (with some exceptions), whereas shallow-water stations have generally higher values. The highest illustrated values are between 4.4 and 8.2 mg/L (stations M2 and M1, respectively, September 2007) with other concentrations commonly exceeding 0.2 mg/L. Stream values can also be high with values commonly exceeding 0.2 mg/L with a maximum value of 1.5 mg/L (PD-in, August 2007).

Abundance of Fecal Microbes

E. coli counts vary temporally and areally over Wilgreen Lake in 2006 and 2007 (Borowski and Albright 2007; Aguiar 2009). In the majority of cases (95 of 123, 77%), *E. coli* counts are below 235 cfu/100 mL (Figure 8) and are therefore *suitable for bathing* as determined by the EPA (2004b). In 12 cases (9.7%), *E. coli* counts are deemed *suitable for recreation only* (236 to 574 cfu/100 mL; EPA 2004b), and in 16 cases (13%) counts specify *no human contact recommended* (>575 cfu/100 mL, EPA 2004b). The highest *E. coli* counts were 5199, 4813, 3921, and 3683 cfu/100 mL at stations TF-1, M1, M2, and TF-in, respectively (Table 3). High fecal microbe counts are not distributed evenly across the lake (Figures 8 and 9) and the relatively low incidence of fecal microbe

pollution suggests that contamination is episodic.

Timing of Fecal Microbe Outbreaks

Rainfall events should sweep fecal microbes into stream and lake waters (e.g., Geldreich 1972; Kleinheinz et al. 2009). We have daily rainfall information from the City of Richmond at its sewage treatment plants of Dreaming Creek and Tates Creek. These locations are outside of the lake's drainage basin, about 3.5 miles to the northeast and 2.5 miles to the north-northeast of the lake, respectively, so any recorded (or unrecorded) rainfall may not represent rain conditions within the Wilgreen watershed. Moreover, summer rainfall in the form of thundershowers is notoriously local and spotty so our characterization of rainfall is not ideal.

The highest *E. coli* counts (*no human contact recommended*) take place on 26 and 30 June 2007 in the shallow waters of Taylor Fork and within inflowing streams (Figure 9). No appreciable rain took place for two weeks prior to rainfall occurring on 25 June (0.35 in., Dreaming Creek; 0.15 in., Tates Creek; Aguiar 2009) and this circumstance prompted us to sample on 26 June. Taylor Fork was flowing, Old Town Branch was trickling, and Pond Cove did not flow with its water only occurring in isolated pools. *E. coli* counts at all of the shallow-water stations of Taylor Fork (Figure 9C) exceeded 2419 cfu/100 mL so for complete quantification we also sampled on 30 June and counted diluted samples. These samples showed counts of up to 5199 cfu/100 mL (Figure 9D), and rain fell on 28 June (0.50 in., Tates Creek) and 29 June (0.75 in., Dreaming Creek; 0.15 in., Tates Creek) before this sample date.

Three to four rainfall events took place between our 30 June and 17 July samplings recording up to 1.35 in. (9 July, Dreaming Creek) but rainfall was insufficient to cause flow in the streams of Pond Cove and Old Town Branch on 17 July. We infer that any rainfall during this time interval did not sweep microbes into the lake to affect microbial counts. *E. coli* counts on 17 July (Figure 9E) exceed the designation of *bathing acceptable*.

Significant rainfall took place on 28–29 July (0.75 in., Dreaming Creek; 2.26 in., Tates

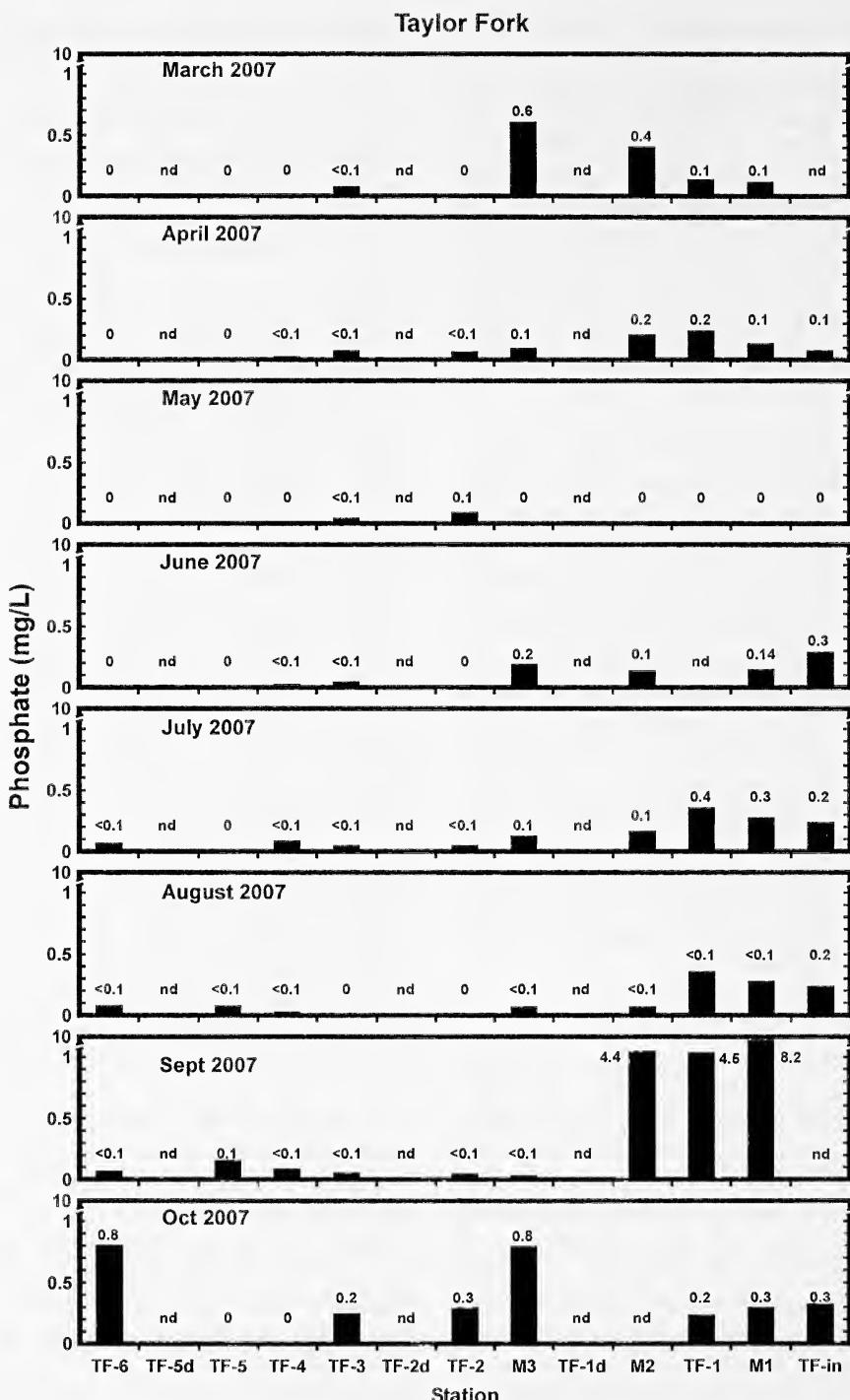


Figure 7. Graphs of phosphate concentration within surface waters through 2007 for Taylor Fork (TF), Old Town Branch (OTB) and Pond Cove (PD) areas of Wilgreen Lake. Water depth decreases from left to right on each panel as stations become more proximal to lake inputs; see Figure 1B for station locations. Note that the concentration scale breaks between 1 and 10 mg/L to clearly show the lower concentrations, and that concentrations are often in excess of that recommended for natural waters (0.05 mg/L PO₄; MPCA 2010). The numbers with the bars indicate phosphate concentration (milligrams/liter, mg/L); nd equals no data. Data from Hunter and Borowski (2008); Aguiar (2009).

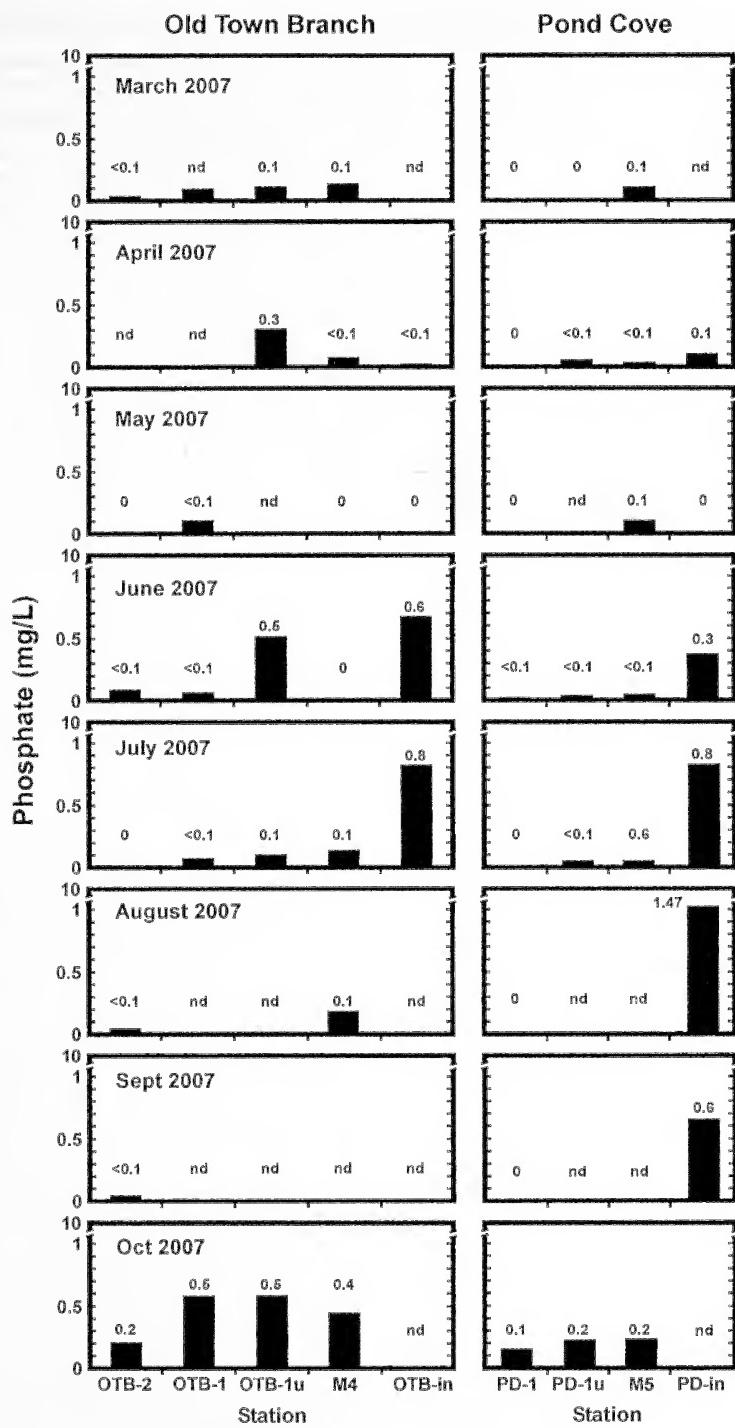


Figure 7. Continued.

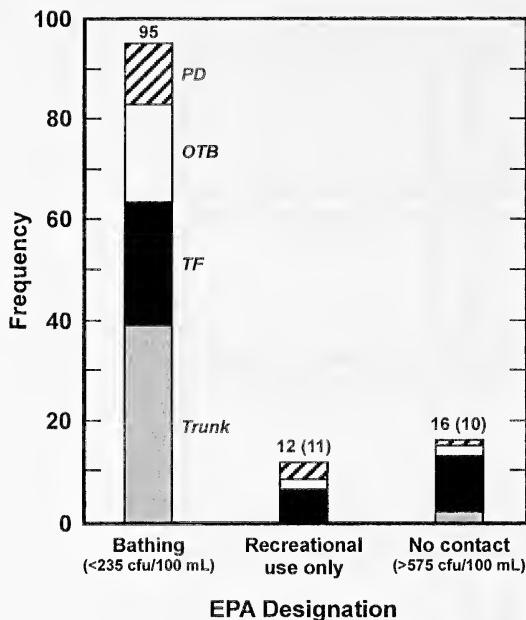


Figure 8. Histogram showing the frequency of *E. coli* counts related to EPA designations for human use: *bathing acceptable* ($<235 \text{ cfu}/100 \text{ mL}$), *recreational use only* (236–574 cfu/100 mL), and *no human contact recommended* ($>575 \text{ cfu}/100 \text{ mL}$) and, which are also related station location (trunk, gray; TF = Taylor Fork, black; OTB = Old Town Branch, white; PD = Pond Cove, striped pattern). See the text for definition of trunk versus shallow-water stations. Some counts exceeding the limit of the IDEXX methods ($>2419.6 \text{ cfu}/100 \text{ mL}$) were resampled and diluted to achieve fully quantitative values; these were samples first taken on 27 July 2007 with resampling on 30 July 2007. Numbers in parentheses refer to total number of samples in the class that were not replicated. See Table 3 for a listing of samples falling in the latter two categories. Data from Borowski and Albright (2007); Aguiar (2009).

Creek) between our microbial sampling dates of 17 July and 1 August. Several stations in the shallow-water portion of Taylor Fork had *E. coli* counts above the *for recreation only* designation; station PD-in had the highest count, exceeding *human contact not recommended* (Figure 9F).

Rainfall occurring between the sampling dates of 1 and 15 August was sporadic in the area. No significant rainfall occurred at Dreaming Creek but 0.9 in. was recorded at Tates Creek on 5 August. All *E. coli* counts excepting that of station OTB-in are below the *for recreation only* designation.

The relationship between rainfall and microbial counts is equivocal. We cannot demonstrate

that high microbial counts within Wilgreen Lake are preceded by rainfall and runoff events. Our highest occurrences of *E. coli* counts exceeding the *human contact not recommended* designation did indeed occur after a rainfall event the preceding day. Apparently, significant rainfall (25, 28, and 29 June) also preceded very high counts recorded on 30 June but water levels within inflowing streams did not rise, suggesting that either appreciable rainfall did not occur within these watersheds and/or rain was insufficient to raise stream levels, perhaps because rain was immediately absorbed by dry soil preventing runoff. One could interpret that this rainfall did not affect microbial counts on 30 June, or alternatively that these highest counts occurred because of the rainfall. On other occasions, rainfall did occur prior to sampling but far enough in advance so that that *E. coli* counts could have spiked immediately after rainfall only to decline to levels documented by measurements. This uncertainty as to the timing of high microbe counts is also pertinent to the question of persistence of *E. coli* within lake waters.

Persistence of *E. coli*

Our data from the muddy environs of Wilgreen Lake suggest that *E. coli* can persist at moderate to high concentrations for 5 to 18 days. In the shallow waters of Taylor Fork, extremely high counts ($>2419 \text{ cfu}/100 \text{ mL}$) can continue in lake waters for at least 5 days (26 to 30 June 2007; Figure 9, panels C and D). Note that counts from Taylor Fork stream (TF-in) are also high, although lower than the highest counts at several other stations (M1, TF-1, M2). From these peak microbe counts on 30 June, *E. coli* counts decreased by a factor of 15 to 164 in the shallow waters and stream of Taylor Fork over an 18-day period to 17 July. The next sampling period (17 July to 1 August 2007; Figure 9, panels E and F) show apparent persistence of moderately high counts over 16 days, but then the subsequent period (1 to 15 August 2007; Figure 9, panels E and F) saw declines by a factor of 1.4 to 8 times over 15 days. These estimates assume that no new injection of fecal microbes from rain events took place between samplings. As we saw in the section above, periods between microbial sampling dates often experienced rainfall so that *E. coli* may have declined over

Table 3. Highest recorded *E. coli* counts in Wilgreen Lake segregated by EPA designation: *recreational use only* and *no human contact recommended* (EPA 2004b). Two sampling sessions occurred in 2006 (24 July, 6 August) and 4 sampling sessions took place in 2007 (27 June, 17 July, 1 August, 15 August). Note that some samples taken on 27 June 2007 exceeded the ability of the IDEXX method in obtaining absolute counts; these stations were re-sampled on 30 June 2007 and diluted to obtain the tabulated absolute counts. Data from Borowski and Albright (2007); Aguiar (2009).

Sample date	Recreational use only ¹			No contact human recommended ¹		
	Sample	<i>E. coli</i> count (cfu/100 mL)	Sample date	Sample	<i>E. coli</i> count (cfu/100 mL)	Re-sampled ² <i>E. coli</i> (cfu/100 mL)
6 August 2006	M2	235	27 June 2007	M3	687	—
17 July 2007	TF-in	243	27 June 2007	TF-2d	816	—
1 August 2007	TF-1	243	1 August 2007	PD-in *	1259	—
1 August 2007	M1	243	27 June 2007	TF-2	1299	—
17 July 2007	TF-1	279	27 June 2007	TF-1d	>2419	1642
6 August 2006	TF-1	290	27 June 2007	OTB-in ^	>2419	2068
1 August 2007	TF-in ^	299	27 June 2007	TF-in ^	>2419	3683
15 August 2007	OTB-in ^	389	27 June 2007	M2	>2419	3921
17 July 2007	PD-in *	399	27 June 2007	M1	>2419	4813
27 June 2007	M4	410	27 June 2007	TF-1	>2419	5199
27 June 2007	PD-in *	488				

¹ Designations from EPA (2004b).

² High counts from 27 June 2007 were re-sampled on 30 June and diluted to produce absolute counts.

* No flow in stream.

^ Low flow in stream.

the entire interval between sampling, or declined then rose again after rainfall only to decline before the next sampling date.

High *E. coli* counts within Taylor Fork proximal to potential septic sources and stream inflow can persist whereas other localities show steeper decreases in microbe counts. Stations TF-2 and TF-2d show declines of 95% and 94% between 26 to 30 June over five days. Likewise over the same period, counts at station M3 decrease 90% from moderate (686 cfu/100 mL) to low levels (70 cfu/100 mL). These stations are distal to stream and potential septic input at the upper reaches of Taylor Fork. Counts seem to remain high at locations close to potential sources but decline more rapidly at stations away from these sources.

Our data show that high *E. coli* counts may persist for as long as 16 days but can also show marked declines over the same period. This suggests that new or continued input of fecal microbes is necessary for any persistence of moderate to high counts. Unfortunately, we cannot demonstrate this supposition because we cannot conclusively document the relationship of high *E. coli* counts to the timing and amounts of rainfall in the watershed. Nevertheless, distal stations (TF-2 and TF-2d, 26–30 June) can show large declines while no decreases are observed proximal to potential fecal sources. In addition, because steep

declines in *E. coli* counts do occur over the period of 30 June to 17 July, we infer that *E. coli* can persist in the natural waters of Wilgreen Lake only for about 3 weeks, assuming no new addition of microbes. Similar persistence times of about 30 days have documented by Wcislo and Chrost (2000) in lake water, and about 28 days in sandy, marine sediment by Craig *et al.* (2004).

Distribution of High Fecal Microbe Counts

High *E. coli* counts most frequently occur in the shallow waters of Taylor Fork (stations TF-1d, -1, -in, M1, M2, and M3) followed by the shallow waters of Old Town Branch (OTB-1u, -in, and M4) and Pond Cove (PD-in) (Figure 9) (Table 3). Of the 10 highest counts above the EPA *no human contact* standard (not counting replicates), six cases occur at Taylor Fork stations (Table 3), proximal to the clustered septic systems of Deacon Hills and Idylwild (letter A, Figure 1B). Other stations from trunk sites, Old Town Branch, and Pond Cove have 2, 1, and 1 occurrences, respectively. Of the 21 cases exceeding the EPA bathing standard, 13 (62%), 3(14%), 3 (14%), and 2 (9%) cases occur at stations of Taylor Fork, Old Town Branch, Pond Cove, and trunk locations, respectively (Table 3). Stations with these high microbial counts include incoming streams in 38% of the cases (eight of 21), but seven of

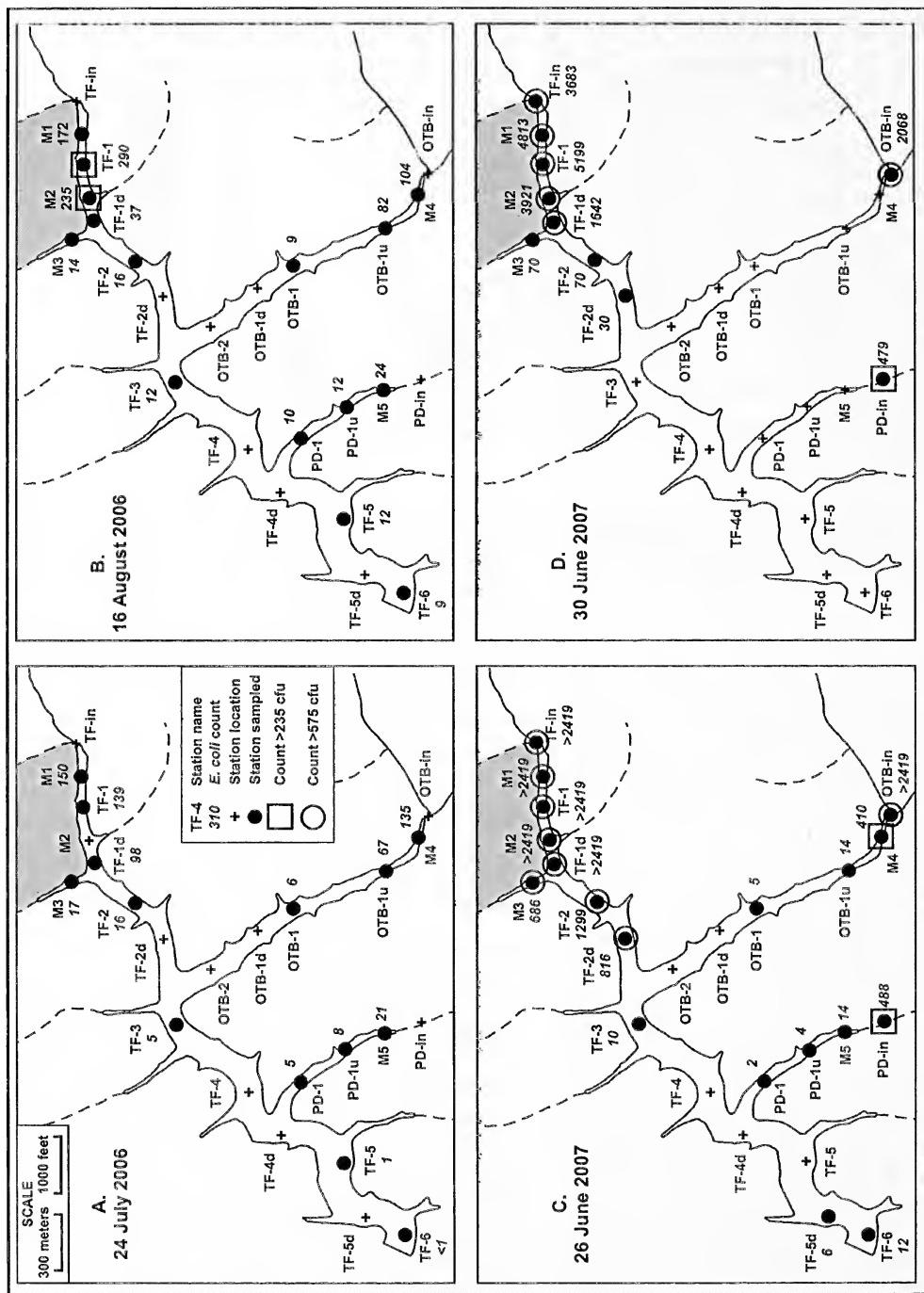


Figure 9. Maps showing the spatial occurrence of *E. coli* counts across Wilgreen Lake for two sampling sessions in 2006 (24 July, 16 August) and five sampling episodes in 2007 (26 June, 30 June, 17 July, 1 August, 15 August). Stations where microbial sampling took place are represented by filled circles and numbers are counts in colony forming units per 100 milliliters ($\text{cfu}/100 \text{ mL}$). Stations with *E. coli* counts exceeding the EPA recommendation for *no human contact* ($>575 \text{ cfu}/100 \text{ mL}$; EPA 2004b) are highlighted with large, open squares. The shaded, gray area in each map shows the location of clustered septic systems within the Deacon Hills/Schindel development. Data from Borowsky and Albright (2007); Aguiar (2009).

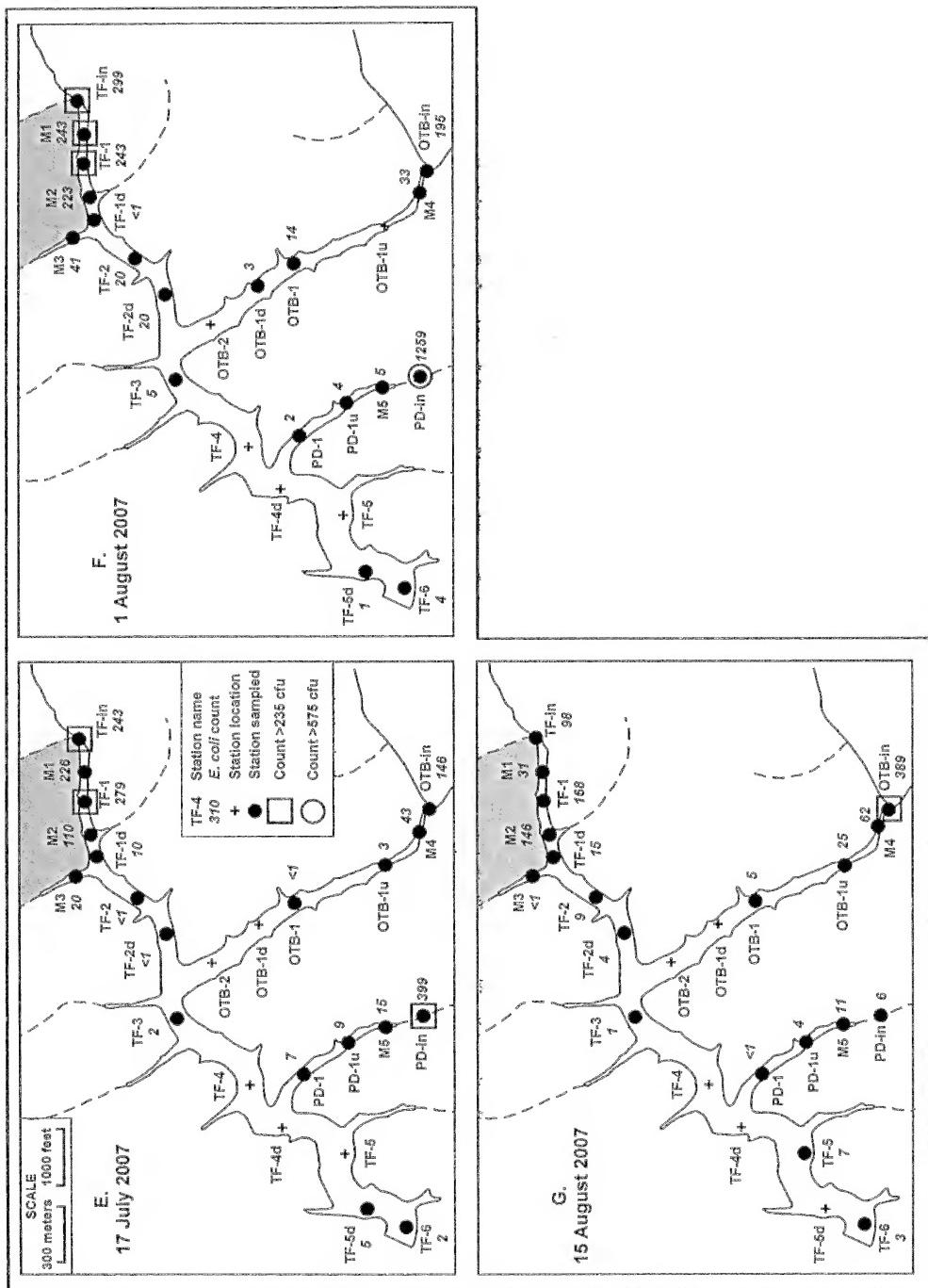


Figure 9. Continued.

eight of these occurrences happened when the streams were stagnant and drying up due to no rainfall.

Most of the lake is unaffected by high fecal microbial counts most of time, so fecal microbial contamination occurs only at discrete times. In 2006, none of the sampling stations showed *E. coli* counts higher than 575 cfu/100 mL, and only 2 stations (16 August 2006; TF-1 and M2) had counts within the *recreation only* designation by the EPA (EPA 2004b) (Figure 8; Table 3). In 2007, only samples taken on 26 and 30 June had samples exceeding the *no human contact* designation (Figure 8; Table 3). On 26 June all the shallow-water, Taylor Fork stations had the highest counts as did 2 of the trunk stations (TF-2, -2d) and OTB-in (Figure 9). We replicated sampling on 30 June to fully quantify the *E. coli* counts and to see how microbial patterns of distribution and abundance may have changed after these several days. High microbial counts still exceeded the *no human contact* designation at Taylor Fork and OTB-in, but *E. coli* counts decreased markedly to 70, 70, and 30 cfu/100 mL at M3, TF-2d, and TF-2, respectively (Figure 9). Thereafter, only PD-in (1 August) had high counts, and only stations in the upper reaches of Taylor Fork, PD-in, and OTB-in had *E. coli* counts at the *recreation only* standard (Figure 9).

DISCUSSION

Use of Nutrient Concentrations and Fecal Microbes as Source Indicators

Patterns of the distribution and abundance of high nutrient concentrations and *E. coli* counts should suggest sources for these contaminants. Dissolved nutrients are produced by decomposition of organic matter and can originate within lake waters or within its drainage basin. Any natural terrestrial habitat (woodlands, fields, etc.) can provide nutrients to natural waters as they become dissolved within rainwater and enter lakes from runoff and groundwater, but anthropogenic sources typically are responsible for severe eutrophication (e.g., Dubrovsky et al. 2010). Because Wilgreen Lake is homogeneous in terms of its physical and chemical properties, we use anomalous concentrations of dissolved nutrients as a source tracking parameter. Higher nutrient concentrations over the propensity of

the lake are segregated within deep-waters trapped below surface and near-surface waters by density differences across the thermocline. Surface waters over most of the lake contain few nutrients because of uptake by photosynthesizers so only where nutrient supply exceeds uptake will nutrients accumulate in surface waters. Recognizing increased delivery of nutrients may identify plausible sources. Still, using anomalous nutrient concentrations to identify specific sources can be problematical because such sources may be numerous, because nutrient reservoirs are replete in natural systems (e.g., soils and sediments) especially those affected by humans, and because dissolved nutrients are mobile chemical species.

An additional tracer is fecal microbe occurrence within Wilgreen Lake. We link the occurrence of high nutrient concentrations and high fecal microbe counts because fecal matter contains enteric microbes and organic matter in varying stages of decomposition including nutrients that immediately or ultimately become dissolved in natural waters.

Use of *E. coli* as an indicator for sources of fecal and nutrient pollution depends on its reliability as a tracer. *E. coli* are enteric, facultative anaerobes (EPA 1986) that can persist in natural aquatic environments to some degree although their abundance typically declines with time (e.g., Crane and Moore 1986). Evidence suggests that predation by protozoans (Korhonen and Martikallon 1991; Hartz et al. 2008) and sunlight (Chandran and Hatha 2005) are significant factors in the removal of *E. coli* from the environment; however, other studies have shown that these gut microbes can persist in refugia of sandy sediments (Craig et al. 2004) and can even grow within natural waters under favorable natural conditions (Carrillo et al. 1985). Thus, whereas governmental authorities like the EPA use *E. coli* counts as indicators of water quality (EPA 1986), the persistence of these microbes in natural waters complicates source tracking. Nevertheless we use the areal distribution of anomalously high nutrient concentrations in surface waters and *E. coli* counts to suggest plausible nutrient sources responsible for the eutrophication of Wilgreen Lake. Using anomalous nutrient concentrations and fecal microbe as

tracers potentially allows identification of the two major sources of pollution in Wilgreen Lake (EPA 2010, 2012), so that results should also suggest remediation steps in improving the lake's water quality.

Areal Pattern of High Nutrient Concentrations in Surface Waters

Deep lake waters generally have higher nutrient concentrations because any nutrients released by decomposition are not utilized by photosynthesizers below the photic zone (e.g., Wetzel 1975). Shallow waters with high nutrient concentrations indicate that nutrient production or delivery is greater than uptake and may point to anthropogenic nutrient input. Excepting deep-waters (depth >2 m), the highest levels of ammonium and phosphate are consistently found at the shallow-water stations of Wilgreen Lake. Of the top 25 measurements of ammonium concentration, 72% are found at either shallow-water stations or incoming streams (Figure 10); of the top 50 measurements, 62% are found at either shallow-water stations or incoming streams. Of the top 25 measurements of phosphate concentration, 80% are found at either shallow-water stations or incoming streams (Figure 10); of the top 50 measurements, 70% are found at either shallow-water stations or incoming streams.

Of the shallow-water stations, the highest nutrient concentrations occur at Taylor Fork (Figure 10). Regarding ammonium and phosphate, 36% of the top-25 nutrient measurements occur in Taylor Fork samples. The next highest group of samples occurs at trunk stations, but these instances generally occur in deeper rather than surface samples. Old Town Branch samples correspond to 20% and 28% of the highest nutrient measurements for ammonium and phosphate, whereas Pond Cove samples for both nutrients represent 16% of the occurrences. High concentrations also occur in incoming streams indicating that the watershed does contribute significant amounts of nutrients to the lake. Stations OTB-in, PD-in, and TF-in have 2, 4, and 1 occurrences of high nutrient levels, respectively (Figure 10). Figure 7 also shows that stream waters contain appreciable amounts of nutrients.

These findings suggest high nutrient concentrations are sourced from either septic effluent entering through groundwater and/or direct runoff into the lake; and/or from streams draining their watersheds. We next examine *E. coli* data for added clues concerning nutrient sources.

Fecal Microbe and Nutrient Sources

High fecal microbe counts are generally restricted to the shallow waters of Taylor Fork, Old Town Branch, and Pond Cove and to their entry streams (Table 3, Figure 9). Moreover the clearest problem with fecal microbe pollution occurs at Taylor Fork, proximal to the concentrated septic systems in housing developments there (Figure 1B, letter A; Figure 9). The close spacing of residences with septic systems is a salient difference between settings of the 3 tributaries entering Wilgreen Lake.

Old Town Branch/Pond Cove. Shallow-water stations and streams of Old Town Branch and Pond Cove commonly contain high (*human contact not recommended*) and moderate (*for recreation only*) *E. coli* counts (Table 3, Figure 9), as well as high nutrient concentrations (Figures 7, 10). Stream waters are mostly responsible for these higher counts when water flow dwindled and the streams eventually ran dry in 2007. Of the lake stations, only station M4 shows moderate counts during these dry conditions (26 June 2007, Figure 9C).

When rainfall occurs and streams regain flow, they must contribute their load of fecal microbes to the lake; however, lake waters within these tributaries have low counts. We posit that once microbes are swept out into the lake, their numbers are diluted by lake waters because additional fecal sources from lake margins are lacking unlike the situation at Taylor Fork.

The drainage basin of Pond Cove is dominated by cattle pastureland, although some dwellings occur within the watershed directly positioned on the stream (outside the map area shown in Figure 1B). The drainage basin of Old Town Branch likewise is dominated by pastureland but comparatively more residences occur, albeit they tend to be widely spaced unlike those within the developments adjacent to Taylor Fork. Some housing

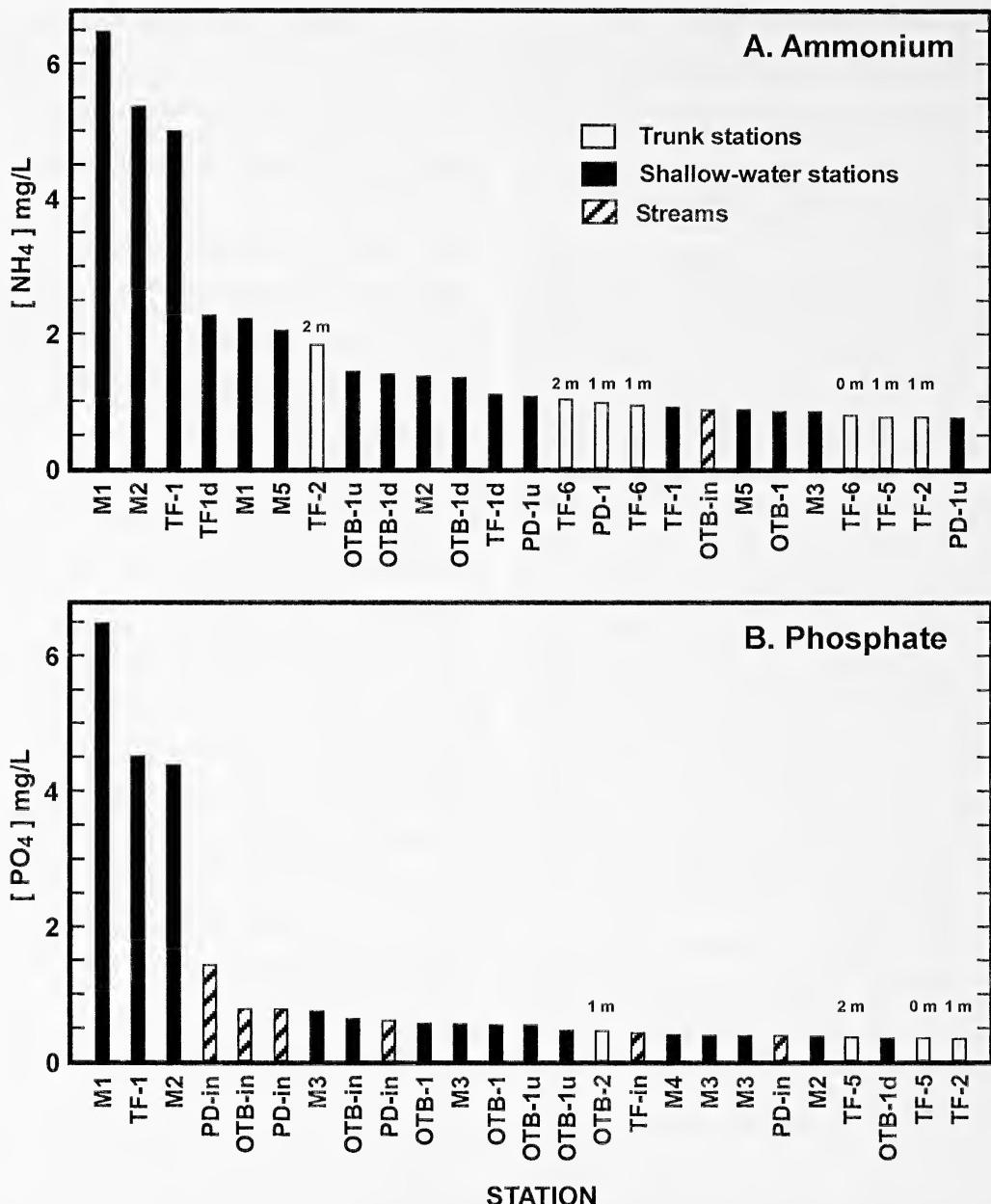


Figure 10. Graphs showing the highest 25 values of dissolved ammonium (A) and phosphate (B) in surface and near-surface (≤ 2 m) waters of Wilgreen Lake. Bars are coded to correspond to trunk stations (white), shallow-water stations (black), and inflowing streams (striped) of the Taylor Fork (TF), Old Town Branch (OTB), and Pond Cove (PD) portions of the lake. Labels with columns refer to the water depth of samples (e.g., 1 m); all other samples are surface samples. See Figure 1B for station locations and the text for definition of trunk versus shallow-water stations. Data from Jolly and Borowski (2007), Hunter and Borowski (2008), Stockwell and Borowski (2008), and Aguiar (2009).

developments on septic systems do occur in the drainage basin (see southeast portion, Figure 1B). Nutrients and fecal microbes from cattle manure may enter the lake through overland runoff, through groundwa-

ter especially in karstic conduits, and through direct deposits into the lake (cattle do enter the lake to drink).

Because pastureland is much more common and because dwellings on septic systems

are so widely spaced, we infer that fecal sources in these watersheds most likely emanate from cattle, although human sources are still a possibility. Because fecal microbe counts of stations more distal from stream inputs (stations M5, PD-1u, OTB-1u, -1, -1d) tend to have very low counts, we also infer that direct runoff from pastureland and dwellings ringing lake margins is less significant as source of nutrients and microbes.

Taylor Fork stream waters. Microbes and nutrients carried by stream water entering Wilgreen Lake through Taylor Fork can come from several possible sources. The drainage basin of Taylor Fork is dominated by industrial, urban, and limited residential use without agricultural inputs (Figure 1). Only residential uses in the form of septic tanks or leaking and broken sewage lines could introduce fecal microbes into Taylor Fork stream, assuming that pet feces are not a significant source. Cattle manure or fertilizer use likewise should not contribute nutrients, also residential fertilizer use remains a possible source. The residences upgradient of the lake are generally on city sewer although some small developments are on septic systems. Thus, high fecal microbe counts within the stream (station TF-in) are consistent with either septic leachate or with sewage entering the stream from leaky sewer lines.

Another potential fecal microbe and nutrient source is from the sewage pumping station, located on Taylor Fork immediately adjacent to the lake (Figure 1B, letter B). At times of high water due to heavy rains, the sewage at the pump station may overflow into the stream and enter Wilgreen Lake. The City of Richmond operates the pump station and must report sewage releases; however, these episodes are the exception rather than the rule. For example, sewage overflows occurred on 14 April, 26 November, and 10 December during 2007 but not during the summer sampling season. No such overflows took place during our sampling times in 2006 either, so we may eliminate this potential fecal microbe source as affecting our data, especially because *E. coli* seem to persist in the lake for about 30 days or less. Although fecal microbes and human sewage

certainly enters the lake via this mechanism, it is likely a small and episodic contributor to the lake system as a whole.

Taylor Fork lake waters. Nutrients and fecal microbes may also be sourced from pastureland and dwellings served by septic systems ringing Wilgreen Lake. Pastureland is limited along the shallow portions of Taylor Fork and cattle are prevented from entering lake waters upstream of station TF-1d by fencing, so we infer that cattle manure is not a significant source of nutrients and fecal microbes here.

Septic systems serving the residential area of Deacon Hills and Idylwild (letter A, Figure 1B) are another possible source of nutrients and fecal microbes. The septic systems are deployed in limestones that produce clayey soils and subsurface karst, and are thus prone to poor functioning. Evidence of septic malfunction includes fetid odors within the neighborhood that also occur right at the lake's edge indicating that sewage is leaking to the surface. A number of small runnels dissect the neighborhood adjacent to Taylor Fork and flow downhill into the lake and thus could directly inject nutrients and fecal microbes into the lake from surface flow. Contribution through the subsurface is also possible by conventional groundwater flow and by flow through karst conduits. Thus it is plausible that sewage containing nutrients and microbes enters lake waters of Taylor Fork upstream of and including station TF-1d.

A Natural Experiment

A drought occurring in summer 2007 provides a natural experiment in ascertaining nutrient and fecal microbe sources. Little rain fell during the summer months and entry streams ran dry (Aguiar 2009). In addition, many of the shallow-water stations of Wilgreen Lake were either dry or inaccessible by boat because of low water levels. Sampling at this time (12 September) established that phosphate (Figure 11) and ammonium levels were elevated only in the shallow-water stations of Taylor Fork. Dry stream beds and lack of rainfall eliminated two possible sources of nutrient delivery to the lake: that from direct runoff from lake margins or contributions from streams. The only other

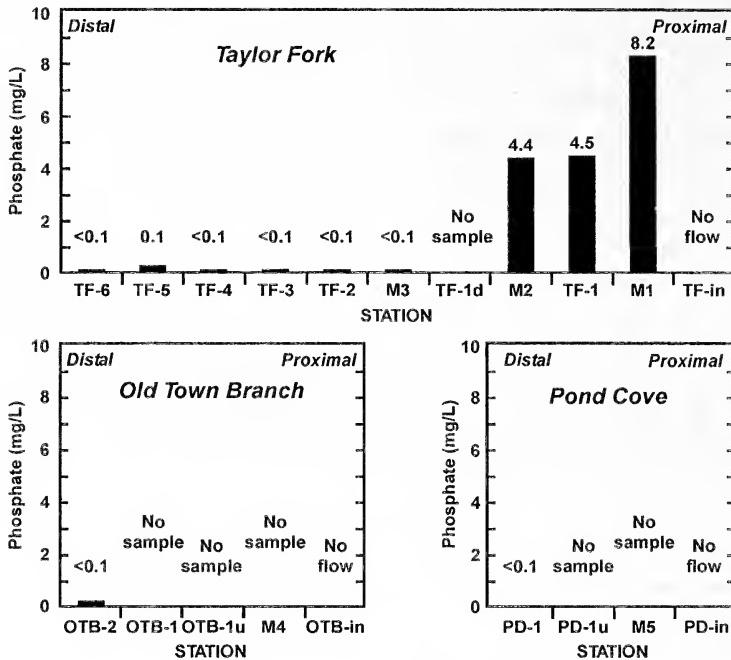
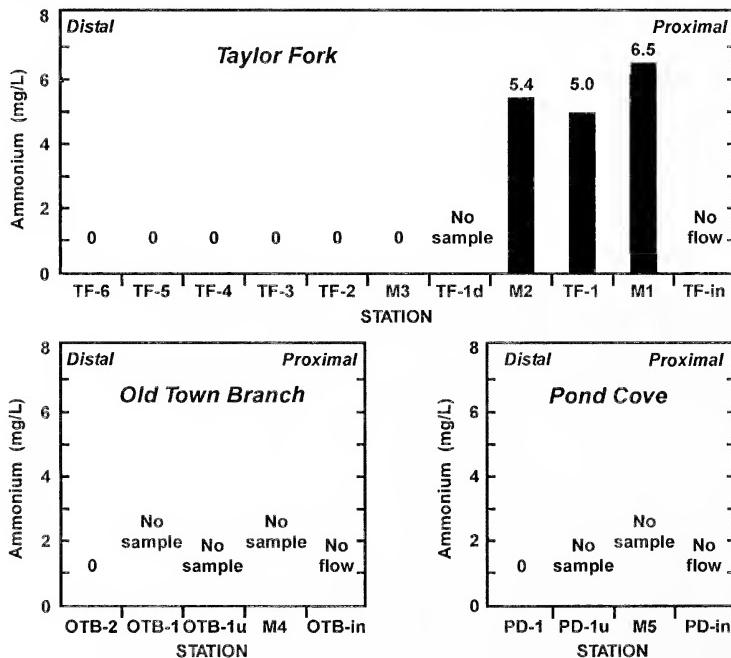
Phosphate - September 2007**Ammonium - September 2007**

Figure 11. Graphs of phosphate and ammonium concentration within surface waters in September 2007 for the Taylor Fork (TF), Old Town Branch (OTB) and Pond Cove (PD) areas of Wilgreen Lake. Due to lack of rain, the lake had a very low water level and entry streams ceased to flow. Note that the highest dissolved nutrient concentrations are located at the shallowest portions of Taylor Fork, proximal to the developments (see Figure 1B, letter A) that possess clustered septic systems. See caption of Figure 7 for further explanation. Data from Hunter and Borowski (2008); Aguiar (2009).

sources for dissolved nutrients are from *in situ* decomposition of organic matter or from entry of groundwater. Sediment analyses from shallow-water sediments typically show low organic matter content (Aguiar 2009), leaving groundwater influx from the septic fields as the most likely source of nutrients during the drought. During normal periods, nutrient sources must also include run-off from streams and lake margins, but our natural experiment demonstrates that nutrients do enter the lake through groundwater. The source of nutrients within groundwater may not be solely from septic drainage, but effluent is a potential concentrated source for nutrients and consistent with our findings; however, we are unable to quantify the proportion of nutrient contribution from each plausible source.

Further Work

Circumstantial evidence above suggests that nutrient and fecal microbe pollution is associated with septic systems around the lake, especially those in the Deacon Hills/Idylwild development (Letter A, Figure 1B) in the shallow reaches of Taylor Fork. Pastureland containing cattle is the other likely source. Nutrients occur concomitantly with fecal sources and will enter the lake along with fecal material. Although human sewage is clearly a source for nutrients and fecal microbes, we cannot quantify the proportion of human versus cattle contributions to lake waters. High nutrient levels, and microbial counts of total coliform and *E. coli*, although indicators of fecal sources (EPA 1986), do not specify host sources (Layton *et al.* 2006). Remediation efforts designed to control nutrient and fecal pollution must be targeted to specific sources to be effective. For example, if cattle manure is the dominate source for nutrients and fecal microbes within Wilgreen Lake, installation of costly sewage lines within Deacon Hills and Idylwild (estimated at \$15K to \$20K per household, 2005), although environmentally desirable, may be unnecessary to halt or decrease eutrophication. To effectively remediate nutrient and fecal microbe pollution effectively in Wilgreen Lake, the relative contributions from humans versus cattle must be determined. We plan to use nitrogen isotopes as

tracer for nutrients (e.g., Silva *et al.* 2001) and PCR techniques utilizing the enteric obligate anaerobe, *Bacteroides* (e.g., Bernhard and Field 2000; Layton *et al.* 2006), to quantify these two most likely pollution sources.

SUMMARY

Circumstantial evidence in the form of increased nutrient concentrations and *E. coli* counts proximal to clustered septic systems in Wilgreen Lake suggest that septic systems are a significant source of nutrients and fecal microbes. However, these contaminants enter the lake through different mechanisms. Nutrients associated with the clustered septic system apparently enter the lake through groundwater, whereas fecal microbes enter the lake through runoff. Other sources for both contaminants are stream inputs and runoff from pastureland surrounding the lake. We cannot yet quantitatively access the individual contribution of these nutrient sources to Wilgreen Lake. We plan to use nitrogen isotopes and PCR assay techniques to distinguish the main sources of nutrients and fecal microbes.

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NOTE

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